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# ON THE PROBABILITY OF FIXATION OF RECIPROCAL TRANSLOCATIONS

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IN a previous paper (Wright, 1940), the following statement was made: "Such mutations as reciprocal translocations that are very strongly selected against until half fixed seem to require some such mechanism to become established. There is an exceedingly deep valley in the surface $\overline{W}$, representing the mean adaptive value in populations with given frequencies of old and new chromosomes and the term $(\overline{W})^{2N}$ in the formula for the joint chromosome frequencies is so small, where $N$ consists of more than some half-dozen individuals, that fixation is virtually impossible." The mechanism referred to was subdivision of at least a portion of the range of the species into isolated territories in which the populations are liable to frequent extinction, with restoration from the progeny of a few stray migrants. The discussion was restricted to forms with exclusive sexual reproduction.

Mr. E. Novitski, in a personal communication, has raised a question as to whether this applies to translocations whose heterozygous unbalanced products may be viable and fertile. These conditions may apply to translocations of the type observed by Dobzhansky and Tan in hybrids between *Drosophila miranda* and *D. pseudoobscura* (to which reference was made in connection with the above statement) (*cf.* discussion by Sturtevant, 1938). The "half-dozen" above was in fact based on assumptions that are unduly severe in this case. Moreover, on re-

examination, I find that the method used was not adequate even for the case considered. The purpose of the present note is to attempt to correct the misleading impression given by this statement.

The probability of fixation of an individual mutation is obtained most directly from the formula, $f(q)$, for the distribution of gene frequencies ($q$) under irreversible mutation. Approximately half of the genes with frequency $1/2N$ (a single heterozygous mutant in a population of effective size $N$) are immediate mutations. Approximately half of those with frequency $(1-1/2N)$ become fixed in the next generation. The chance of fixation is accordingly $f(1-1/2N)/f(1/2N)$. In the absence of selection, the formula for the frequencies of populations in relation to $q$ is $2v/q$, where $v$ is the mutation rate of the gene per generation. The number of mutations per generation is thus $(1/2)f(1/2N) = 2Nv$, and the number fixed per generation is $(1/2)f(1-1/2N) = v$. The chance of fixation of a single mutation is thus $1/2N$. Unfortunately the formula for the distribution under irreversible mutation has been worked out only for exceedingly low selection coefficients apart from the special case in which there is no dominance (review in Wright, 1938).

The formula for the distribution at equilibrium under reversible mutation has been worked out more generally (Wright, 1937, 1938). For the case in which mutation rates in both directions are indefinitely low, it is $C(\overline{W})^{2N}/q(1-q)$ where $\overline{W}$ is the mean adaptive value for the population. This distribution may be looked upon as the sum of the distributions for irreversible mutation in both directions, weighted so that the number of genes fixed in both directions is the same. The chance of fixation of a mutation can not be obtained directly from this formula, but a rough idea can be got from the ratio of the minimal ordinate to the subterminal one at $q = 1/2N$. Thus if a mutation must pass through a class with frequency only $10^{-20}$ of that of the class for which $q = 1/2N$, before reaching fixation ($q = 1$) it is obvious that the chance of fixation

must be low. This does not give the basis for an accurate estimate, however, unless account can be taken of the rate at which genes pass through the class in question.

This may be made clearer by returning to the case of irreversible mutation. We will assume that the mutation is consistently advantageous, having an advantage $s$ over type when heterozygous and $2s$ when homozygous. The distribution takes the form $C(1-exp[-4Ns(1-q)])/q(1-q)$ where $C$ is a constant, $2v/[1-exp(-4Ns)]$. The rate of fixation as deduced from the ratio $f(1/2N)/f(1-1/2N)$ is approximately $2s$ if $Ns$ is large (Fisher, 1930, Wright, 1931). At the mid-point of the distribution, the class frequency $f(1/2)$, is approximately $8v$. The rate of change of gene frequency is maximum at this point: $\Delta q = sq(1-q) = s/4$. The average number of classes that are carried past the mid-class by selection in each generation may be obtained by dividing by the class interval, $1/2N$, and is thus $Ns/2$. The number of genes carried past this point is obtained by multiplying by the mid-ordinate and is thus $4Nsv$, not allowing for differences in the ordinates in this neighborhood. Variations in $\Delta q$, due to accidents of sampling, will approximately cancel each other if the coefficient of variability of $\Delta q$ is small. As the number of mutations is again $(1/2)f(1/2N)$ or $2Nv$, the estimate of the proportion of the mutations that are carried past the mid-point is $4Nsv/2Nv$ or $2s$. But with the strong selection postulated, there can be no appreciable return of such genes, so that this figure, $2s$, should also be the approximate chance of fixation. It may be noted that this agrees with the direct estimate from the subterminal classes.

The situation is somewhat more complicated at points where there is no selection pressure. In the distribution $2v/q$, the frequency of the mid-class, $f/(1/2)$, is $4v$. This class is scattered in both directions in the next generation with a standard deviation, $\sigma_{\Delta q} = \sqrt{q(1-q)/2N} = \sqrt{1/8N}$. The values of $q$ increase by steps of $1/2N$ or $\sigma_{\Delta q}\sqrt{2/N}$ in this neighborhood. Thus the portion of the mid-class

that is carried to higher values of $q$ is approximately $(.5 - prf\ [.5\sqrt{2/N}])$ where $prf$ is the probability function. The portion $(.5 - prf\ [1.5\sqrt{2/N}])$ of the class below the mid-class, $q = [.5-(1/2N)]$, is carried beyond the mid-class and so on. For $N = 20$, the total number of genes carried beyond the mid-class comes out $1.37f(1/2N)$ while $1.12f(1/2N)$ are carried across the same point from the reverse direction. The net flux is thus $.25f(1/2N) = v$ from which it may be deduced that the average rate of fixation per generation is $v$ and the chance of fixation of an individual mutation is $v/2Nv = 1/2N$, again in agreement with the estimate from the subterminal frequencies. For the case $N = 50$, $2.11f(1/2)$ are carried past the mid-class toward fixation and $1.86f(1/2)$ are carried in the opposite direction, again giving a net flux of $.25f(1/2)$ or $v$. It is to be noted that the total flux in both directions ($2.48f(1/2)$ and $3.97f(1/2)$ in the cases above) is not a very large multiple of the net flux. This ratio should vary approximately as the standard deviation of sampling and hence may be represented as $1.6\sqrt{2N}$.

We may now consider the chance of fixation of reciprocal translocations. This involves the complication that two chromosomes are involved, but in the simplest case, that of semisterility in plants, this is wholly obviated. Only two types of gametophytes are viable ($AB$ and $A'B'$) and only three kinds of zygotes are produced. In a population producing the array of viable gametophytes $[(1-q)AB + qA'B']$, random union of gametes results in zygotes according to the terms of $[(1-q)AB + qA'B']^2$ exactly as with a single pair of alleles. The case can thus be treated exactly as if only a single pair of alleles were involved with semisterility of the heterozygotes. It will be assumed here that the adaptive value ($W$) of the zygotes is according to the relative number of viable gametophytes that they produce, an assumption that is not necessarily correct, since the disadvantage of semisterility may be partially offset by the reduced competition among progeny of the same plant.

| *Zygote* | *Frequency* | *Selective value (W)* |
|---|---|---|
| AABB | $(1-q)^2$ | 1 |
| AA′BB′ | $2q(1-q)$ | ½ |
| A′A′B′B′ | $q^2$ | 1 |

Thus $\overline{W} = 1-q(1-q)$ and $f(q) = C[1-q(1-q)]^{2N}/q(1-q)$. As there is symmetry in the action of selection, above and below the mid-class, this distribution may be thought of as composed of the sum of two similar distributions for irreversible mutation in opposite directions. The frequency at $q = 1/2N$ would be substantially the same in one of the latter distributions as in the symmetrical distribution, the frequency at $q = 1/2$ would be just half that of the symmetrical distribution, while that at $q = (1-1/2N)$ is unknown but very small. Thus for irreversible mutation, the ratio of the frequency of the mid-class to the number of mutations per generation is $(1/2)f(1/2)/(1/2)f(1/2N)$ where $f$ refers to the formula for reversible mutation. This ratio is approximately $(2e/N)(3/4)^{2N}$ except for very small values of $N$.

We have seen that if there is no selection at all, the chance of fixation of a mutation is one fourth of this ratio. In the case under consideration, there is no selection pressure at the mid-class, but the slope of the distribution is steeper than where there is no selection at any point, and the net flux is correspondingly greater. Exactly how much greater has not been demonstrated, but as we have found that the total flux in both directions across the mid-point, due to accidents of sampling, is only a small multiple (about $1.6\sqrt{2N}$) of the net flux, in the absence of selection pressure, the chance of fixation in small populations can not be either higher or lower than the above ratio by a factor that would lead to seriously misleading conclusions. On this basis, the chance of fixation of a translocation that is semisterile when heterozygous is between one fourth and $0.4\sqrt{2N}$ of $1.6 \times 10^{-3}$ if the effective size of population is 10, of $2.7 \times 10^{-6}$ if $N$ is 20 and of $3.5 \times 10^{-14}$ if $N$ is 50.

The situation is different in animals in which it is probable that there is no selective elimination in the haploid phase (Muller and Settles, 1927). We will consider first the case in which only the balanced types survive long enough to leave offspring but will assume that these types are all equal in viability.

| Zygotes | Frequencies | | | | | |
|---|---|---|---|---|---|---|
| | Parents | Gametes | | | | Offspring |
| | | $AB$ | $AB'$ | $A'B$ | $A'B'$ | |
| $AABB$ | $x$ | $x$ | | | | $[x+(y/4)]^2$ |
| $AA'BB'$ | $y$ | $y/4$ | $y/4$ | $y/4$ | $y/4$ | $2[x+(y/4)][(y/4)+z]+y^2/8$ |
| $A'A'B'B'$ | $z$ | | | | $z$ | $[(y/4)+z]^2$ |
| Total | 1 | $(x+(y/4))$ | $y/4$ | $y/4$ | $((y/4)+z)$ | $[x+(y/2)+z]^2+y^2/8$ |

As long as most of the population consists of one of the homozygous types, the situation does not differ substantially from that in semisterile plants. Only a small proportion $(y/2)$ of the gametes of the homozygotes takes part in inviable unions, and slightly more than half $[(1/2)+(y/8)]$ of the gametes of heterozygotes do this. There is, however, always somewhat less selection, relatively, against the heterozygotes than in semisterile plants. This difference is greatest at the point at which $AB$ and $A'B'$ have become equally numerous. At this point the frequencies of the zygotes are .2347 $AABB$: .5306 $AA'BB'$ : .2347 $A'A'B'B'$ instead of .25 : .50 : .25. The value of $\overline{W}$ at this point is .7826 instead of .75. The chance that a translocation of this sort will become fixed is thus somewhat greater than in the case of semisterile translocations of plants.

Next it is desirable to consider the case in which the heterozygous unbalanced types are as viable as the balanced types and in which therefore only the homozygous deficiencies are eliminated in each generation. In this case it is necessary to deal with two-dimensional frequency surfaces. Unfortunately, the formula of the surface that fully meets all the conditions is not known. A preliminary estimate may, however, be obtained.

Let $p$ be the frequency of chromosome $A'$ and $q$ that of $B'$ in a population. We will assume (not quite correctly) that the frequencies of the two affected chromosomes are combined at random in the zygotes, before elimination of $AAB'B'$ and $A'A'BB$. The joint frequencies are then according to the terms of $[(1-p)A + pA']^2[(1-q)B + qB']^2$. Under reversible mutation from $A$ to $A'$ and from $B$ to $B'$ at indefinitely low rates in both directions, the formula for the distribution is as follows:

$$f(p, q) = C(\overline{W})^{2N}/p(1-p)q(1-q) \text{ where } \overline{W} = [1-p^2(1-q)^2 - (1-p)^2q^2]$$

The surface is the same if $AB$ mutates reversibly, by reciprocal translocation, to $A'B'$ and $AB'$ mutates reversibly to $A'B$, all at indefinitely low rates.

This surface is saddle-shaped with indefinitely high class frequencies at (0, 0) and (1, 1), and a deep valley along the transverse diagonal, $p = (1-q)$, with zero class frequencies at its ends, (0, 1) and (1, 0). A population passing from homallelic $AABB$ to homallelic $A'A'B'B'$ must cross this valley at some point. In estimating the chance that this may occur it is necessary to consider the class frequencies along this diagonal and not merely that of the mid-class (1/2, 1/2). With the assumptions made above, $\overline{W} = 7/8$ for the mid-class, and $f(1/2, 1/2) = 16C(.875)^{2N}$. The sum of the class frequencies along the diagonal would be $2N$ times this value if all classes were equally frequent. The actual value can be estimated by constructing the frequency curve $f(p, 1-p) = C[1-p^4 - (1-p)^4]^{2N}/p^2(1-p)^2$. It turns out that the factor by which $f(1/2, 1/2)$ is to be multiplied is 6.4 if $N = 20$, 9.7 if $N = 50$, 13.4 if $N = 100$ and 18.9 if $N = 200$ instead of $2N$ in each case.

The ratio of this sum of class frequencies along the line of half fixation to the frequency $f(1/2N, 1/2N) = 4N^2C$, of populations that are homallelic $AB$ except for one representative of $A'B'$, should give a fraction of the same order of magnitude as the chance of fixation of a single translocation. This ratio is $3 \times 10^{-4}$ if $N = 20$, $2.5 \times 10^{-8}$ if $N = 50$, $1.4 \times 10^{-14}$ if $N = 100$ and $1.2 \times 10^{-26}$ if $N = 200$.

These, however, underestimate the chance of fixation because of the assumption that the affected chromosomes are combined at random. A population that is not in equilibrium with respect to the combination of two pairs of alleles returns only 50 per cent. toward random combination in each generation (Robbins, 1918). Thus the elimination of $AAB'B'$ and $A'A'BB$ should result in a correlation in each population. At the point (1/2, 1/2) the frequencies of the zygotes before elimination of $AAB'B'$ and $A'A'BB$ are easily found to be as follows, from consideration of the frequencies of the gametes and of the products of random union among them.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $AABB$ | .0891 | $AA'BB$ | .1203 | $A'A'BB$ | .0406 | $BB$ | .2500 |
| $AABB'$ | .1203 | $AA'BB'$ | .2594 | $A'A'BB'$ | .1203 | $BB'$ | .5000 |
| $AAB'B'$ | .0406 | $AA'B'B'$ | .1203 | $A'A'B'B'$ | .0891 | $B'B'$ | .2500 |
| $AA$ | .2500 | $AA'$ | .5000 | $A'A'$ | .2500 | | 1.0000 |

The adaptive value ( $\overline{W}$ ) of this population is .9188 ($1-2\times.0406$) instead of .875 as assumed above. The factor by which $f(1/2, 1/2)$ is to be multiplied would be increased slightly and $f(1/2N, 1/2N)$ would be slightly reduced. Fortunately the chance of fixation falls off so rapidly with increase in the effective size of population that even a factor of error of 10 is of little importance except in the case of very small numbers. Replacing $(.875)^{2N}$ in calculating $f(1/2, 1/2)$ but making no other corrections, the ratio of the sum of population frequencies along the line of half fixation to $f(1/2N, 1/2N)$ is about $3\times10^{-3}$ if $N=20$, $4\times10^{-6}$ if $N=50$, $3\times10^{-10}$ if $N=100$ and $5\times10^{-18}$ if $N=200$. The chance of fixation of a reciprocal translocation on which selection acts only by eliminating the homozygous unbalanced types should be of the order of the above figures in the sense used in the case of semisterility. In the case $N=20$, it may be noted that the chance of joint fixation of two indifferent mutations ($1/4N^2=6\times10^{-4}$) is less than one fourth the number given above. The reciprocal translocations have a certain advantage as far as joint fixation is concerned, though the impossibility of separate fixation which more than

compensates for the effects of adverse selection where $N$ is as small as 20. For $N = 50$, however, two indifferent mutations have more than 100 times as much chance of joint fixation as the translocations.

Finally, it should be noted that the probability of fixation of a chromosome change is enormously greater if one of the unbalanced homozygotes is viable and fertile (as might well be the case in a small insertional translocation). There would, in fact, be a minute selection in favor of such a translocation as long as the complementary chromosome is present.

## Summary

The chance of fixation of a reciprocal translocation in a population of plants, with exclusive sexual reproduction, is of the order $10^{-3}$ if the effective population number ($N$) is 10. It is of the order of $2 \times 10^{-6}$ in groups of 20 individuals and of the order $3 \times 10^{-14}$ in groups of 50 individuals. It is assumed that the heterozygotes are semisterile, and that there is no compensating advantage in semisterility by reduction of competition among the progeny and that the translocation has no advantage *per se*. These figures may be compared with $1/2N$, the chance of fixation of an indifferent mutation.

Reciprocal translocations in animals have a slightly better chance of fixation than in plants in populations of the same effective size, even if only the balanced types are viable and fertile.

Cases in which the heterozygous unbalanced type are at no disadvantage in viability and in number of gametes produced, only the homozygous deficiencies being eliminated, have considerably better chances of fixation than in the cases above. The chance is roughly $3 \times 10^{-3}$ in populations of 20, $4 \times 10^{-6}$ in populations of 50, $3 \times 10^{-10}$ in populations of 100 and $5 \times 10^{-18}$ in populations of 200. In all the cases given here, there is an element of uncertainty as a result of which the true chance may be smaller or greater by a small factor (less than 4).

A statement in a previous paper gave a somewhat exaggerated impression of the difficulty of fixation of reciprocal translocations. It remains true, nevertheless, that such fixation can hardly occur under exclusive sexual reproduction except in a species in which there are numerous isolated populations that pass through phases of extreme reduction of numbers. The most favorable case would seem to be that in which there is frequent extinction of the populations of small isolated localities, with restoration from the progeny of occasional stray migrants from other localities.

If one of the unbalanced homozygotes is viable and fertile (unlikely in a reciprocal translocation but not unlikely in the case of a small insertional translocation), the chance of fixation of the nondeficient modified chromosome may be much greater than in the cases considered above and would even be slightly greater than that of an indifferent mutation if the unbalanced homozygote and the heterozygotes that include it are at no disadvantage compared with the balanced homozygotes.

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# THE VIRILIS GROUP OF DROSOPHILA IN TEXAS

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## INTRODUCTION

RECENT studies have shown that the species commonly called *Drosophila virilis* Sturtevant exhibits a series of variant forms which apparently represent distinct steps in evolutionary progress (Spencer, 1938, 1940; Patterson, Stone and Griffen, 1939, 1940). It is for this reason that any new facts relating to this species is of general interest to the biologist.

Spencer (1940) has recently brought together the collection records from America for two of the subspecies, *D. virilis virilis* and *D. virilis americana.* He lists three records for the first of these two subspecies from the United States. Two of these had been recorded by Sturtevant (1921), a pair of flies taken in New York City by him in 1913, and a strain from a grocery store obtained by Mr. R. R. Hyde in Terre Haute, Indiana. The third record consists of a specimen taken in Los Angeles, California, and mentioned by Metz, Moses and Mason (1923). In addition to these three records, there is another stock which was derived from one or more flies taken in New Orleans, Louisiana.

The second subspecies, *D. virilis americana,* was discovered by Spencer, who captured a male and female in Wayne County, Ohio, in 1936. Two years later, he and Mr. Harrison Stalker captured three females at Gatlinburg, Tennessee (Spencer, 1940). He mentions what probably constitutes an earlier record for this form. This is a damaged specimen in the pinned collection of Professor A. H. Sturtevant. It was taken many years ago near Chattanooga, Tennessee, by Mr. W. S. Adkins. It is known that other specimens of this subspecies have been taken in the last couple of years, but since these

records have not been published, we shall not consider them further.

It is the purpose of this article to supplement the records listed by Professor Spencer by our own from Texas. During the past two and a half years we have collected in Texas a total of 185 individuals belonging to the *virilis* group.

## COLLECTION RECORDS FROM TEXAS

In Table 1 are given the complete records for these 185

TABLE 1

DATA ON COLLECTIONS OF *virilis* IN TEXAS

| Gray form | Red form | Date | County | Place | Habitat |
|---|---|---|---|---|---|
| | 3 | Aug. 12, 1940 | Anderson | Palestine | Woods, Roadside Park |
| | 1 | Aug. 9, 1940 | Bell | Salado | Woods, Roadside Park |
| | 1 | Aug. 23, 1940 | Bell | Belton | City Park |
| 1 | | Dec. 11, 1939 | Bexar | San Antonio | Produce house |
| 34 | | June–July, 1940 | Bexar | San Antonio | Produce houses |
| | 1 | Sept. 30, 1938 | Blanco | Johnson City | Near store, trap |
| 2 | | July 27, 1939 | Blanco | Blanco | Grocery store |
| | 1 | June 11, 1939 | Cherokee | Jacksonville | Woods, tomato dump |
| 1 | | June 24, 1940 | Dallas | Dallas | Produce house |
| 10 | | Nov.–Dec., 1939 | Galveston | Galveston | Produce house |
| 7 | | July 16, 1940 | Galveston | Galveston | Produce house |
| 8 | | June 18, 1940 | Harris | Houston | Produce houses |
| 3 | | Sept. 15, 1938 | Hays | Henly | Grocery store |
| 2 | | July 3, 1940 | Jefferson | Beaumont | Produce house |
| | 1 | Oct. 25, 1940 | Liberty | Devers | Woods, Roadside Park |
| | 2 | Aug. 14, 1940 | Newton | Bon Wier | Swamp-thicket |
| | 1 | April 4, 1939 | Tarrant | Fort Worth | Backyard, trap |
| 5 | | May–June, 1940 | Tarrant | Fort Worth | Produce house |
| 1 | | Nov. 15, 1938 | Travis | Aldrich | Woods, trap |
| 1 | | May 5, 1939 | Travis | Aldrich | Woods, trap |
| | 1 | Feb. 16, 1940 | Travis | Aldrich | Woods, trap |
| 1 | | Aug. 17, 1940 | Travis | Aldrich | Woods, trap |
| 1 | | Sept. 9, 1940 | Travis | Dessau | Woods, sweep-net |
| 84 | | July, 1940 | Travis | Austin | Produce house |
| 1 | | Nov. 20, 1938 | Victoria | Victoria | Fruit store |
| 6 | | May–June, 1940 | Victoria | Victoria | Fruit store |
| | 1 | Sept. 13, 1938 | Williamson | Georgetown | San Gabriel Park |
| | 3 | Aug. 9, 1940 | Williamson | Georgetown | San Gabriel Park |
| | 1 | Aug. 9, 1940 | Williamson | Round Rock | Woods, Roadside Park |
| **168** | **17** | | | | |

specimens. They are listed under two types, the gray forms and the red forms. These terms refer to the color of the pupae of these two groups of flies. In the first type the pupae are black or gray, while in a new subspecies found in Texas in 1938 and named *D. virilis texana* (Patterson MS.), the pupae are red. *D. virilis americana* Spencer also has red pupae, and in addition, *americana* and *texana* have a number of other characters in common. The most obvious difference between them

is in the character of the chromosome complex, a fact that will be discussed in a later section of the paper. We refer to these two groups as gray and red forms because cytological and genetic studies have shown that the different strains of each type are not homogeneous (Patterson, Stone and Griffen, 1940).

In addition to recording the two types, the table also gives the date of capture, the county, the city in (or nearest) which the specimens were taken and the nature of the habitat. In all except four instances the exact date of capture is given. The four exceptions represent cases where several flies were collected at the same place on different occasions and in adjacent months. Thus, by the way of illustration, a total of thirty-four flies was captured in the produce houses of San Antonio on three trips made during the months of June and July, 1940.

On the basis of the records, the gray form would appear to be very much more common in Texas than the red form. A total of 168 gray forms was collected as against seventeen reds. Of perhaps greater significance is the nature of the habitats in which the two forms are found. Almost without exception the gray form was taken in towns and cities, that is, in fruit and grocery stores and in produce houses. It therefore lives in what may be termed a "domestic habitat." There were but four exceptions to this rule. Three different individuals, collected at different times, were taken in the woods from traps at the Aldrich Place in Travis County. The fourth specimen was taken in sweepings by Mr. Robert Wagner near Dessau, which is also located in Travis County.

In contrast to this, the red forms were all captured in the woods. They therefore live in what may be called a wild or "natural habitat." However, two individuals were taken within city limits. The first of these was the fly captured at Johnson City on September 30, 1938. It was caught in a trap placed in a lot back of a grocery store. Johnson City is a town of about 350 inhabitants, and the lot where the trap was placed contains trees and

is adjacent to the open country. The second specimen is the one collected at Fort Worth on April 4, 1939. It was captured in a trap by Mr. W. E. Briles in the back yard of his home. This home is located at the eastern edge of the city where there are tree-covered vacant lots.

It was stated above that the gray form appears to be more common in the state than the red, and, so far as numbers are concerned, this is true. But if the data are analyzed from the point of view of localized breeding colonies, this assumption is not so obvious. The basis for an analysis of this sort is shown in Table 2.

TABLE 2

NUMBER OF PLACES AT WHICH THE TWO FORMS OF *virilis* WERE TAKEN

| Gray forms | | | Red forms | |
|---|---|---|---|---|
| San Antonio, | Produce houses | 35 | Palestine, refuse can Roadside Park | 3 |
| Houston, | " " | 8 | Georgetown, refuse can S. G. Park | 4 |
| Austin, | Produce house | 84 | Belton, refuse can City Park | 1 |
| Galveston, | " " | 17 | Salado, " " Roadside Park | 1 |
| Forth Worth, | " " | 5 | Devers, refuse can Roadside Park | 1 |
| Beaumont, | " " | 2 | Round Rock, refuse can Roadside Park. | 1 |
| Dallas, | " " | 1 | Jacksonville, tomato dump | 1 |
| Victoria, | Fruit store | 7 | Bon Wier, thicket-swamp, trap | 2 |
| Henly, | Grocery store | 3 | Johnson City, trap | 1 |
| Blanco, | " " | 2 | Fort Worth, " | 1 |
| Aldrich, | traps | 3 | Aldrich, " | 1 |
| Dessau, | sweepings | 1 | | |

Specimens of the gray form have been collected at twelve different places in the state. The five produce houses at which the thirty-five specimens were taken in San Antonio are all located on two adjacent city blocks, and the distance between the two most widely separated houses is not over one hundred yards. The breeding population within this small area has ready access to all five of these houses. Likewise, the two produce houses from which we secured specimens at Houston are located directly across the street from each other, thus presenting the same opportunity for the population of this area. It is not necessary to comment on the conditions at the single produce houses at Austin, Galveston, Fort Worth, Beaumont and Dallas, at each of which the gray form was collected. The seven specimens from Victoria were all collected at one fruit store, but on three separate oc-

casions. No other specimens were found in the other stores of that city. The three flies from Henly were caught in a trap, and the two from Blanco were captured on fruit in a store. Those taken at Aldrich and Dessau were commented on above.

The red forms were collected at eleven different places (right side, Table 2), but only the three records consisting of two or more flies require fuller explanation. The three from Palestine were all captured at one time in a single refuse can, and the two from Bon Wier were also taken at the same time and from one trap. The four flies recorded from San Gabriel Park near Georgetown were collected on two different occasions. The first catch was made on September 13, 1938, and consisted of a single fertile female from which the type material for the new subspecies *texana* was developed. This fly was captured in a refuse can. The second catch was made on August 9, 1940, and consisted of three females. All three flies were taken from a single refuse can which was located at almost exactly the same point where the first female was captured.

In the judgment of the writer, the capture of such a rare form for a second time at the same point, after an interval of nearly two years, is not to be regarded as a mere coincidence. We have learned from experience that the kind of habitat in which the red form is most likely to be found is typified by this particular part of San Gabriel Park. It therefore seems worth while to describe this place in some detail. The park is located about a mile from Georgetown in Williamson County. It lies for nearly a mile along the west bank of the San Gabriel River, and is covered by many large forest trees. The grass on the main part of the park is kept short by mowing or by grazing sheep. Directly opposite the point where the four red forms were taken, and at a distance of about one hundred yards, there is a narrow, low-lying strip of land adjacent to the river. This strip is subject to overflows from the river, and its soil is usually water-

logged. No attempt is made to keep this part of the park in formal condition, and during the growing season it is covered with a rank growth of vegetation. Shrubs and grapevines are also present, and the rotting logs and stumps within this area are often covered with growths of fungi. This place constitutes an ideal environment for *Drosophila* species, and it must be here that the red form of *virilis* is breeding. It can not breed in the refuse cans with which the park is supplied for the convenience of the public, for these are thoroughly cleaned out every other day by a park attendant.

The capture of the red and gray forms at only eleven and twelve places, respectively, with only one place in common (Aldrich), would seem to indicate that the two forms represent thin populations. This is undoubtedly true, particularly in view of the fact that we have visited many stores and have trapped at many points in the country. If either form has a dense population, surely by chance alone we would have taken them at many other places.

The contrast in the total numbers taken for the two forms is easily understood if the difference in habitats is taken into consideration. The gray forms usually live in and about stores of various kinds. Produce houses which handle bananas are their favored breeding places. Here, throughout the year, they have an abundance of food, sufficient moisture, constant temperature in the vaults and protection from the elements during extreme weather conditions. They are thus able to breed generation after generation and eventually to build up colonies of comparatively large size. It is interesting to note that of the 168 specimens listed in the table, 152 were collected in produce houses, twelve in grocery stores and only four in the country or woods.

The red forms clearly prefer the country as a place in which to live and breed. Thus far we have not taken a single specimen in stores or produce houses. Moreover, in the country they seem to prefer a rather definite type

of habitat, like the one described at San Gabriel Park. But even in the best of these they are not able to build up as large colonies as their gray relatives. They lead a much more precarious existence than the gray forms, for out in nature they do not have as constant a supply of food and are exposed to the extremes of weather conditions. Nevertheless, the red form has available many more suitable breeding places than has the gray form. It is physically impossible for the collector to search all such places; the best that he can do is to sample some of these potential breeding habitats. On the whole, the breeding colonies seem to be small, but what they lack in size may be made up for by numbers. Hence the total number of red specimens in a given area may exceed the total number of gray specimens.

### Percentage of Virilis in the Population of Drosophila

It is not possible to take a complete census of any wild species living in nature. But it is sometimes possible to obtain a random sample of a group of species, and thus to determine the ratio of any one of these to the other members of the population. In the case of *Drosophila* species even this is difficult. There is no way of being certain that the collecting methods used will secure the different species in numbers approximating their exact proportion in the population, no matter how large the sample. In trapping insects like *Drosophila* the collector has no way of knowing whether individuals of the various species are being attracted to the baited trap with equal ease. If they are not attracted with equal ease, the sample will not be a random one. This possibility must be kept in mind in considering the percentages of *virilis*-like forms in our sample.

Up to the present time we have collected and classified a total of 610,804 specimens belonging to the genus *Drosophila*. On the basis of these figures the 168 gray forms give a ratio of one gray fly to about every thirty-six hundred (3,635) specimens in the sample, and the

seventeen red forms give a ratio of one in about every thirty-six thousand (35,929) specimens.

As will be shown in the next section, the distribution area of the *virilis* group does not cover the entire state, while our sample does. The percentages based on the total catch at the places where the different forms of *virilis* were captured will give some idea of their frequency in the population in these restricted areas. These percentages are displayed in Table 3. Both types show

TABLE 3

PER CENT. OF *virilis*-LIKE FORMS AT POINTS OF CAPTURE

| Place | Gray forms | | | Place | Red forms | | |
|---|---|---|---|---|---|---|---|
| | Total catch | G-forms | Per cent. | | Total catch | R-forms | Per cent. |
| Aldrich ..... | 130,456 | 3 | 0.002 | Aldrich ..... | 130,456 | 1 | 0.001– |
| Austin ..... | 1,032 | 84 | 8.14 | Belton ..... | 403 | 1 | 0.25 |
| Beaumont ... | 1,017 | 2 | 0.19 | Bon Wier .... | 875 | 2 | 0.23 |
| Blanco ..... | 2,333 | 2 | 0.08 | Devers ..... | 703 | 1 | 0.14 |
| Dallas ..... | 2,981 | 1 | 0.03 | Fort Worth .. | 1,864 | 1 | 0.05 |
| Dessau ..... | 751 | 1 | 0.13 | Georgetown .. | 7,014 | 4 | 0.06 |
| Fort Worth . | 12,045 | 5 | 0.04 | Jacksonville . | 11,572 | 1 | 0.009 |
| Galveston ... | 6,620 | 17 | 0.26 | Johnson City . | 1,209 | 1 | 0.08 |
| Henly ..... | 3,828 | 3 | 0.08 | Palestine .... | 1,960 | 3 | 0.15 |
| Houston .... | 3,843 | 8 | 0.21 | Round Rock . | 1,081 | 1 | 0.09 |
| San Antonio . | 16,544 | 35 | 0.21 | Salado ..... | 2,046 | 1 | 0.05 |
| Victoria .... | 1,140 | 7 | 0.61 | | | | |

considerable variation in the percentages for different places. For the gray forms it varies from two thousandths of one per cent. at the Aldrich Place to above eight per cent. at the Austin produce house. The percentages for four of the produce houses (Beaumont, Galveston, Houston, San Antonio) are of the same order of magnitude, with a difference of only 0.07 per cent. between the two extremes.

Among the red forms the Aldrich Place again gives the lowest per cent., while Belton shows the highest. On the average the gray forms give a much higher per cent. than the red forms. If these percentages represent a fair measure of the frequency of the two forms in the populations in their respective habitats, they support the contention made above regarding the difference in size of the breeding colonies.

### Geographical Distribution

The gray and red forms of *virilis* thus far have been found only in the northeastern part of the state. This area is bounded on the west by the 98th meridian and on the south by the 29th parallel. Both the flora and the fauna of Texas are diverse, as would be expected from its varied topography and climate and its geographic position. There is a conspicuous overlap along the 98th meridian where the humid eastern coniferous and deciduous forests gradually merge into the Central Prairie, which in turn passes into the dry High Plains and their associated vegetation.

The western limit of the range of many eastern species of animals occurs along this overlap. This is true for a fairly large group of Drosophila species which heretofore have been recorded from the Mississippi Valley States. This distribution area has the heaviest rainfall of any part of the state and includes the main timber belt of Texas. The same type of vegetation extends eastward to the Mississippi River and beyond. Since members of the *virilis* complex have been found in Ohio and southward through Tennessee, Alabama, Louisiana and then west into Texas and New Mexico, it seems probable that Texas represents the southern edge of its geographical distribution area. In the northern part of the continent, however, its geographic range is known to extend to the west coast. Recently Mr. G. B. Mainland of this laboratory collected fourteen specimens of the gray form at Santa Barbara, California, thus augmenting the earlier record from Los Angeles.

### Cytology and Genetics of the Virilis Group

#### (*a*) *The gray group*

It has long been known that *D. virilis virilis* Sturtevant has five pairs of rod-shaped and a pair of dot-shaped chromosomes in metaphase. The longest pair of rods represent the sex-chromosomes. All strains of the gray

group thus far studied have this same type of chromosome complex, with but one exception. Chino and Kikkawa (1933) found among their untreated stocks a strain in which chromosomes 3 and 5 were fused to form a V-shaped element.

In our investigations of the southwest group of gray forms we have found that while the chromosome complex is consistently of the *D. virilis virilis* type, yet the several strains show significant genic differences. These differences were brought to light through a series of crosses between strains from the southwest (Texas and Louisiana) and those of China, Japan, *D. virilis virilis* and the subspecies *americana* and *texana*. The results obtained show definitely that the several strains of the gray group which were tested are not homogeneous, and this has led us to subdivide it into *D. virilis virilis,* the Southwest group and the Asiatic group. Subsequent tests with new stocks of the gray group from the Southwest, and from Asia (kindly sent to us by Professor M. Chino), have demonstrated conclusively that even within these subdivisions there are striking differences in genotype. For example, females of several stocks when tested to *americana* males showed that the amount of cross-fertility varies from as low as one per cent. in one stock to as high as fifty-eight per cent. in another stock at the upper end of the series.

### (*b*) *The red group*

The red group consists of *D. virilis americana* of Ohio and *D. virilis texana* of Texas, together with several new red strains from the Southwest. The cytology of *americana* has been studied by Hughes (1939), who has shown that in metaphase the female has a pair of rods, a pair of dots and two pairs of V-shaped chromosomes. In the male there is one pair of rods, a pair of dots, a pair of V's and a single V which is paired with two rods. He showed that one limb of an *americana* X is the homologue of the rod-shaped *virilis* X chromosome, while the other limb is the homologue of a rod-shaped autosome. The

configuration in the male of *americana* is due to the fact that the Y chromosome is not fused with an autosome.

In our own studies on *americana* we have carried the analysis a step further, and have shown that the autosomal V's correspond to the pairs 2 and 3 of the gray group. Moreover, the autosome fused with the X is chromosome 4, and since there is no Y-4 fusion, the male has, in addition to the pair of dots, only three V-shaped chromosomes and four rods (Patterson, Stone and Griffen, 1940).

We have also worked out the cytology of *D. virilis texana.* In this subspecies both males and females in metaphase have one pair of V's, three pairs of rods and a pair of dots. The sex-chromosomes are not involved in the pair of V's. The pair of V-shaped chromosomes in *texana* was found to be the equivalent of two pairs of autosomal rods, fused at the centromeres, and corresponding to chromosomes 3 and 4 of the gray group. Hughes has shown that in the salivary gland chromosomes *americana* differs from *virilis* in the gene arrangement along the chromosome. The order of the genes in *americana* is inverted simply or in complex manner in four of the long chromosomes, the X, 2, 4 and 5. We found that *texana* differs from *virilis* in chromosomes X, 2 and 5, with the simple inversions in 2 and 5 apparently identical with those of 2 and 5 in *americana.* Finally, the *texana* X has only two of the three inversions which cause the difference between *virilis* and *americana.*

We have carried out a series of crosses to test the fertility of the different strains, both between members of the two groups (red and gray) and within each group. It is not the purpose of this article to describe the results obtained from these crosses, for that has been done elsewhere, but a few of the more important results can be considered. Crosses between members of the gray group were found to be quite fertile, while those between *americana* and *texana* were less fertile. Crosses which were least fertile were those between members of the two

groups. Some few of these were only slightly fertile, even after repeated tests were made. None of the crosses between the groups was therefore completely sterile.

There are several factors involved in this high degree of sterility which contribute to the separation of *virilis* into the various semi-isolated groups. In addition to the influence of gene rearrangements caused by inversions and fusions, a very important factor is sexual isolation, or the lack of mating. Sexual isolation was found to be very strong between members of the red and gray groups unless *D. virilis virilis* females are used in the cross. Moreover, females heterozygous for *virilis* and any other member of the gray group are often fertile with males of *texana* and *americana*, but *virilis* males cross poorly with females of either *texana* or *americana*. Another factor is poor egg hatch, which was found to be very low from females known to be fertilized for all crosses tested. A very important isolating factor is a complex interrelated fertility mechanism. This causes sterility of $F_2$, $F_3$, etc., males of both *texana* and *americana*, and is of a genetic character. The effect operates through activities controlled by the Y-chromosome and necessary for fertility in the males. If the *texana* Y is present in hybrids, autosomes 2 and 5 must also be present for the male to be fertile. *Americana* has a similar type of mechanism.

### (*c*) *New stocks of the red group*

In addition to the original stocks of *americana* and *texana*, we now have available several other strains of the red group. Genetic and cytological studies of these new stocks show that they fall into three classes. The first class is represented by a stock derived from a single male. This male was mated to three different types of females, namely, *virilis*, *texana* and *americana*. He was cross-fertile with *americana* but nearly cross-sterile with the other two. A study of the metaphase plates in ganglion cells of the hybrid larvae shows that he had five pairs of rods and one pair of dot-shaped chromosomes,

with no fusions or V-shaped elements. A study of the salivary chromosomes of these larvae shows that, in reference to *americana,* inversions are present in chromosomes 2, 3, 4 and 5. In phenotype this male resembled members of the red group in shape, broadly clouded cross-veins, time of etherization, manner of pupating in the food and in carrying the factor for red pupa color. This form therefore constitutes a new subspecies, to be known hereafter as *Drosophila virilis novamexicana.*

The second class includes nine stocks, all belonging to the *texana*-like type. Five of these represent pure strains, that is, each stock was developed from a single fertile female captured in nature. In each of these strains the gene arrangement is the same as that for *texana,* and a single pair of V's is present in the metaphase plate. In four of the five strains it has already been determined that the fused pair of autosomes is the same as in *texana* (chromosomes 3 and 4). Of the four remaining stocks of this class, two were derived from males and two from females which were virgin at the time of capture. All four of these stocks are cytologically like *texana.* Most of these stocks have been tested, and the results obtained indicate that several of them differ genetically from *texana.* This is true even for some of the pure strain stocks in which no difference in gene arrangement could be detected.

The third class includes two stocks, both derived from females which were virgin at the time of capture. One of the females was mated to *texana* males and the metaphase plate of the $F_1$ larvae contained three V-shaped chromosomes. Since one of the V's came from the father, the other two must have come from the mother, and this would indicate that she was *americana*-like. An examination of the salivary chromosomes supports this conclusion. The other female was mated to *americana* males, and here the metaphase plate of the $F_1$ larvae showed four V-shaped chromosomes, thus indicating that this female was also of the *americana* type. It was de-

termined that chromosomes X, 2, 3 and 4 had the same gene arrangements as the corresponding chromosomes of *americana*. But chromosome 5 was like that of *virilis*, and therefore did not have the same gene arrangement as chromosome 5 in *americana*.

## DISCUSSION

On the basis of the earlier collection records it has been assumed that *virilis* is a very rare species in North America. Our experiences in collecting show that while it represents a "thin population," yet it is by no means uncommon and therefore can not be regarded as one of the rarest forms of *Drosophila* in this country. Moreover, it can not be regarded as a single species, rather, it represents a group complex, which, because of differences in pupa color, can be subdivided into gray and red forms.

We have examined cytologically, and tested genetically, a fairly large sample of gray forms, selected from among more than 150 stocks which were developed from specimens collected here in the southwest and elsewhere. Thus far, no cytological difference has been detected among the several stocks examined. Genic differences, however, do exist. In contrast to this, three subspecies are recognizable among only thirteen available stocks of the red form, and in the two which have more than one stock, both genic and cytological differences are found. A very interesting cytological relation exists between the subspecies (Table 4).

The inversions listed for the salivary chromosomes of the red forms are in reference to those of *virilis*, which is used as the standard. The first point to observe is that chromosome fusions have occurred independently of the inversions and of one another. The relation between the three red subspecies is not a simple one of direct lineal descent. After their separation from the ancestral form, various changes obviously have occurred. These changes must have been accompanied by hybridization,

TABLE 4

SHOWS RELATION OF CHROMOSOMES IN THE THREE SUBSPECIES

| Gray forms | Red forms | | |
|---|---|---|---|
| *Virilis* | *Texana* | *Americana** | *Novamexicana* |
| Chromosome X | Two overlapping inversions | A third inversion superimposed on *texana* type | *Americana* type |
| | fused with | fused with | |
| Chromosome 4 | No inversions | Two included inversions | Single inversion, *americana* type of larger inversion |
| | fused with | | |
| Chromosome 3 | No inversions | No inversions | New single inversion |
| | | fused with | |
| Chromosome 2 | Single inversion Variable | Single inversion, *texana* type | New single inversion |
| Chromosome 5 | None, or one or two independent inversions, depending on strain | Two inversions like those of *texana*, but both always present | Standard |
| Chromosome 6 | Standard | Standard | Standard |

* Y-chromosome not fused in any of the subspecies.

unless one is willing to accept the improbable hypothesis that two or more identical inversions occurred in separate strains. There are several possible ways in which this diversification could have taken place. Of these, the scheme shown in Fig. 1 seems the most probable to the writer and his coworkers, Drs. W. S. Stone and A. B. Griffen.

The difference of the degree of cross-fertility exhibited by many gray and red strains indicates that this variability is due to differences in genotypes. Nevertheless, no two strains are completely cross-sterile, so that complete genic separation has not yet occurred. The presence of so many differences within a crossfertile series suggests that diversification is of rather recent occurrence.

We have shown that if $F_2$ or $F_3$ red-gray hybrid males which have a Y-chromosome from red strain are to be fertile, they must also have certain autosomes from the same source. This relation has been found to be consistent for all red strains tested. Since the autosomes

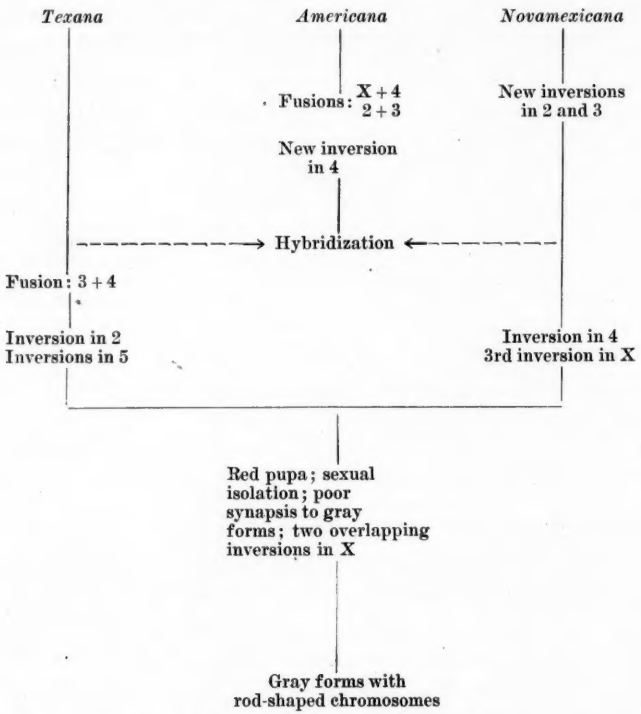


FIG. 1. Diagram showing most probable relationship between subspecies.

in question may be heterozygous, the interrelation must be dependent on dominant genes in the autosomes. In species crosses wherever such Y-autosome interdependence exists, the fertility of the hybrids may be affected. In the *virilis* case the effect can not be detected until the $F_2$ generation. However, in crosses where the autosomal genes are recessive, the $F_1$ male would be sterile. This therefore offers another explanation for certain cases where $F_1$ hybrid males are sterile, although their sisters are fertile.

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# POLYDACTYL FEET OF TWO STRAINS OF CHICKS[1]

PROFESSOR MARY T. HARMAN AND FRANCES NELSON[2]

## Introduction

Two strains of polydactyl chicks are found among the birds belonging to the Poultry Department at Kansas State College. One of these strains, in which the hallux is usually duplicated, arose from a single White Leghorn hen. This strain has been called "duplicate polydactyl." The other strain resulted from matings of selected individuals showing this type of polydactylism and from the regular strain of polydactyl birds. The polydactylism is usually manifested in one long hallux possessing a metatarsal, and either three or four phalanges, instead of the three bones which occur normally. This strain has been designated "polyphalangeal polydactyl."

The purpose of this investigation has been to determine and to classify the variations which occur in these two strains of polydactyl chicks.

## Review of Literature

Observations of the development of the normal foot of chicks have been made by Rosenberg (1873), Morse (1880), Baur (1883), Johnson (1883) and Strong (1920). Strong determined the time and order of ossification of the bones of the normal foot.

Fisher (1935) emphasized the differences found—heterozygous and homozygous polydactyl chicks.

Harman and Alsop (1938) gave an outline of the ossification processes which occur in the feet of polydactyl chicks during embryonic life. They also made some inci-

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dental observations on normal development of the chick foot.

## Material and Methods

A total of 469 feet were studied. Of these, 337 were from the duplicate polydactyl strain and 132 were from the polyphalangeal polydactyl strain. In both strains, embryos and hatched chicks were considered. All the feet were fixed in 95 per cent. alcohol, cleared in a potassium hydroxide solution, and stained with alizarin (Dawson, 1926).

## Observations

In both strains of chicks there were a great number of variations in the feet examined. Furthermore, the variations were such that a graduated series from those most nearly normal to the most abnormal could be arranged. In fact, the two strains graded into each other, which makes it difficult to arrange them into definite classes.

## Duplicate Polydactyl Chicks

Of the 337 feet of the duplicate polydactyl strain, 34 were taken from embryos of from nine to twelve days' incubation. These were not sufficiently ossified to classify. Sixteen feet were definitely normal and six others appeared normal. The remaining 280 feet have been arranged in six classes. The basis for this classification is the shape and development of the metatarsal of the first toe or hallux, and the number of digits associated with this metatarsal, and the character and number of phalanges in these digits.

*Note.*—The term "short" has been applied to a digit when it is composed of a metatarsal and one or two phalanges. The term "long" has been used to describe a digit when it is composed of a metatarsal and more than two phalanges.

*Class I.*—Class I has a long and a short hallux. Each hallux has a separate metatarsal at the base of the first phalanx (Fig. 1, M and $M^1$). The short hallux has two

phalanges. The long hallux, always on the outside, has four phalanges. There were 33 feet of Class I. On two of the feet the shorter hallux had a double last phalanx or claw.

*Class II.*—Class II has two long halluces. One hundred thirty-three of the feet studied belong to this class. Four of these had separate metatarsals. In most of the feet of this class the metatarsal for the halluces was large, and often the proximal phalanx was broad and common to both digits. Fig. 2 is a drawing of a foot of this class. The first metatarsal in this instance is elongate like a cannon bone, M. However, nothing was found that would indicate that this metatarsal was formed like a cannon bone. In 19 feet of Class II each hallux had four phalanges and eleven feet had three phalanges. In 88 feet, the outer hallux had four phalanges and the inner one had three.

In none of these feet did the outer hallux have a smaller number of bones than the inner, although occasionally by actual measurement the outer hallux was shorter than the inner. Fusion or lack of separation was evident in most of these feet. In many instances there was no separation except in distal phalanges.

*Class III.*—Class III has one short and two long halluces. There are 67 feet which belong to this class. Ten of these have only a trace of a third hallux (Fig. 3, 1). Fifty-seven have the short hallux composed of two phalanges. The longer digits are always on the outside. No evidence of a splitting of the shorter digit was found (Fig. 4, 1).

*Class IV.*—Class IV has three long halluces. There are 38 feet in this class. In three feet all the halluces are of the same length. Each has one metatarsal and three phalanges. Twenty-four feet have a single common metatarsal, an outer hallux of four phalanges and two inner ones each composed of three phalanges (Fig. 5, 1, $1^1$ and $1^2$). In 12 feet the two outer halluces are

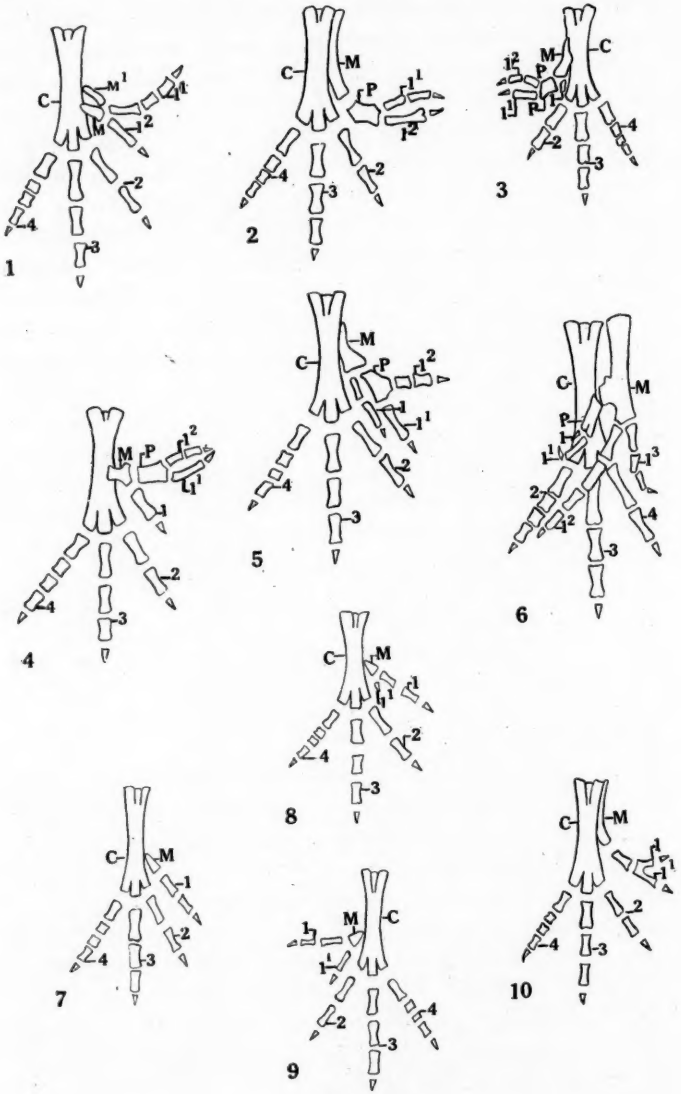
composed of a single common metatarsal and four phalanges, while the inner hallux has three phalanges.

*Class V.*—Class V has four halluces. There are only four feet which belong to this class. A metatarsal is common to all the halluces and the number of phalanges in each hallux ranges from two to five (Fig. 6). In this particular foot, the four halluces seem to have arisen in pairs as indicated by the forking of the large metatarsal, M. One hallux of the inner pair is composed of two phalanges, and the other is composed of three. The proximal phalanx of this pair (P) is common to both halluces but is forked at its distal end. The outer pair of halluces articulate with the same fork of the metatarsal, but the phalanges are entirely separate. The outer hallux has four phalanges and the inner one has five. This was the first instance found in which the outer hallux had fewer phalanges than any of the inner ones. In fact, only three feet were found in which the outer hallux had fewer phalanges than the inner halluces.

*Class VI.*—Class VI has one long hallux composed of a single small metatarsal and three phalanges. Digit 1 of Fig. 7 illustrates this condition. There were five feet of this class found in this strain of chicks. They are similar to the most common type of the polyphalangeal polydactyl strain.

There does not seem to be any regularity in the sizes and shapes of the metatarsals, even within their respective classes. In some feet the metatarsal extends the full length of the cannon bone and is fused to it (Fig. 2 M, C). Other metatarsals of the same length are not fused to the cannon bone. In some instances, the metatarsal is fused to the cannon bone to such an extent that it appears like a branch of it. Some of the metatarsals are small (Figs. 1 and 4). The shape of the metatarsals range from those similar to the normal (Fig. 1) to the large club-shaped bone illustrated in Fig. 6, M.

Considerable variation in the position of the metatarsal of the hallux in relation to the cannon bone was found.




FIGS. 1–10.

Some metatarsals are parallel to the cannon bone; some are at right angles to it; others lie across it. Often the metatarsal lies in a groove in the cannon bone. In some instances, the metatarsal for the hallux is quite large and seems to have crowded the other three metatarsal bones to the extent that the resulting cannon bone is abnormal in shape.

Many specimens have fewer metatarsals than halluces, while others have one metatarsal for each hallux. When two metatarsals are present, they may or may not be of equal size. In some instances, the duplicate metatarsals are entirely separated, while others are united in almost every possible arrangement. Often two slender curved metatarsals are joined at both ends but are separate in the middle. Forked or Y-shaped metatarsals occur.

Seventy-two pairs of the duplicate polydactyl feet show bilateral variation. Eighty-seven pairs do not show bilateral variation. The two classes which have the greatest number of halluces include 23 feet from the left side and 20 from the right. The class which ranks next in complexity includes 36 right feet and 29 left feet.

EXPLANATION OF FIGURES

Fig. 3 was drawn natural size. All other figures were drawn two times natural size. All figures are reduced by one half. Figs. 1 to 6, inclusive, are drawings from the feet of the duplicate polydactyl strain. Figs. 7 to 10, inclusive, are drawings from the feet of the polyphalangeal strain. All the feet were from hatched chicks. The labeling is the same throughout. 1, $1^1$, $1^2$, and $1^3$, halluces; 2, 3, 4, second, third, and fourth digits; M and $M^1$, metatarsal for the halluces; C, cannon bone; P, proximal phalanx for the halluces.

Fig. 1. Left foot of duplicate polydactyl strain of Class I.

Fig. 2. Left foot of duplicate polydactyl strain of Class II.

Fig. 3. Right foot of duplicate polydactyl strain of Class III.

Fig. 4. Left foot of duplicate polydactyl strain of Class III.

Fig. 5. Left foot of duplicate polydactyl strain of Class IV.

Fig. 6. Left foot of duplicate polydactyl strain of Class V.

Fig. 7. Left foot of polyphalangeal polydactyl strain of Class I. The feet of Class VI of the duplicate polydactyl strain are similar to this one also.

Fig. 8. Left foot of polyphalangeal polydactyl strain of Class II.

Fig. 9. Right foot of polyphalangeal polydactyl strain of Class III.

Fig. 10. Left foot of polyphalangeal polydactyl strain of Class III.

### POLYPHALANGEAL POLYDACTYL CHICKS

As was previously stated, the polyphalangeal polydactyl feet are characterized by the presence of more than the usual two phalanges in the hallux. Although the greater number of the feet of this strain examined have one long hallux, some have extra halluces. Based upon the number of halluces present and upon the number of phalanges in each hallux, the 132 feet of the polyphalangeal polydactyl strain examined has been grouped into three classes.

*Class I.*—Class I has a single hallux, with a nearly normal metatarsal and three or four phalanges (Fig. 7). Eighty-five of the feet examined belong to this class. Seventy-six of these have three phalanges, while nine have four.

*Class II.*—Class II has one long and one very short hallux. The short hallux has only one phalanx, and this sometimes is so small that it seems no more than a very small claw (Fig. 8, $1^1$). This claw-like digit is always between the long hallux and the second digit. Sometimes this extra hallux is oval in shape and has the appearance of a small, misplaced metatarsal. Twenty-two feet are in this class.

*Class III.*—Class III has two halluces, each of which is composed of more than two bones (Fig. 9). There were 25 feet in this class. Eight of these had one long and one short hallux, while 17 had two long halluces. In most of the halluces of the latter group, there was only distal separation, while in the former group the two halluces were separated to the metatarsal (Figs. 9 and 10).

More bilateral variation was found in the polyphalangeal polydactyl strain than in the duplicate polydactyl strain. Twenty-three of the 66 pairs of feet studied show bilateral symmetry, while 43 pairs do not.

Of the 208 pairs of feet from both the duplicate polydactyl strain and the polyphalangeal polydactyl strain studied, 106 pairs show bilateral variation; 102 pairs do not.

## Discussion

Among the duplicate polydactyl strain of chicks, there are only five feet with a single hallux composed of a metatarsal and three phalanges. Fisher (1935) reported that this type of foot was rare in the chicks with which he worked. However, in the polyphalangeal strain it was found to be the most common type of polydactylism.

Bond (1920) and Fisher (1935) state that the left side is more often polydactyl than the right side. Results of the studies herein reported seem to indicate that in these two strains polydactylism is as apt to occur on the right side as on the left side.

Neither Fisher (1935) nor Harman and Alsop (1938) reported finding any seven-toed feet. Four such feet are included in this study. Two of these were from the same chick. The other two were paired with less complicated feet.

Whenever there were two or more halluces unequal in length the longer hallux or halluces were usually on the outside. There were only three exceptions to this arrangement found among the 469 feet studied. The pair of feet with the seven digits had the following number of bones in the halluces, given in order from the outside to the inside: Right foot, three, five, three and three; left foot, two, five, four and three. One foot had three halluces, of which the outer hallux had two bones, the middle one five, and the inner one three. The total number of bones within the halluces of a single foot of the duplicate polydactyl strain ranged from three to fourteen. The average number within the hallux of a single foot was eight, although nine bones occurred more often than any other number. Fisher (1935) found the average to be between seven and one half and eight.

The process of ossification and the order of ossification in the feet used in this study were similar to that reported by Harman and Alsop (1938).

The size of the phalanges in the region of the hallux varies extremely as does the arrangement and shape.

Forked or Y-shaped phalanges are common when any degree of union occurs. The pattern varies so greatly that six classes had to be established and even within these classes the pattern was not constant.

There was an intermediate group between the duplicate polydactyl strain and the polyphalangeal strain. Feet similar to the one illustrated by Fig. 7 were found in both strains. However, of the 337 feet of the duplicate polydactyl strain only five were of this type while 85 of the 132 of the polyphalangeal polydactyl strain were of this type. On the other hand, two halluces occurred in 25 feet out of 132 feet of the polyphalangeal polydactyl strain, while 332 out of the 337 feet of the duplicate polydactyl strain had two or more halluces.

## CONCLUSIONS

Although a large amount of variation occurred in both strains of chicks, the feet of the duplicate polydactyl strain were grouped into six classes and those of the polyphalangeal strain were put into three classes, based upon the number and arrangement of halluces in each foot and the number and arrangement of the phalanges composing each hallux.

No consistent bilateral variations were evident in either strain.

Whenever two or more halluces were present the longer hallux was usually on the outside.

Never more than two metatarsals for the halluces of the duplicate polydactyl strain were present, and only one was present in any of the feet of the polyphalangeal polydactyl strain.

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# THE WHALE SHARK UNAFRAID

## THE GREATEST OF THE SHARKS, *RHINEODON TYPUS*, FEARS NOT SHARK, MAN NOR SHIP

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### INTRODUCTION

BARRING the internal parasites which it harbors, the whale shark has no enemies—it literally fears nothing. This may be due in part to its great size—it grows to 60 feet in length—and certainly to its tremendously thick skin. All sharks have thick, solid skins, but the whale shark's is thick beyond all others. Underneath the epidermis in a 32-foot shark is a 4-inch-thick layer of connective tissue as dense, solid and resistant as a solid-rubber truck tire. The whale shark is virtually clad in armor. Sharks have no ribs, and their muscles are attached directly to the skin. This skin in a sense acts as an exoskeleton. Hence, in a huge heavy shark like Rhineodon, the skin must be thick, dense and strong to give form and substance to the great body.

Furthermore, the whale shark is the enemy of no living things except the small invertebrates and fishes upon which it feeds. And since all sharks are omnivorous carnivores, this seems a most extraordinary thing.

All large sharks are provided with large cutting or tearing teeth in proportion to their size and to the kind of prey they use as food. Thus provided they are supposed to be enemies of all other sharks, of all large fishes, and particularly of man. Furthermore, excepting when "hanging around" ships for scraps thrown over from the galley or when feeding on a whale moored alongside a ship for "cutting in," sharks commonly avoid ships.

The shark is always hungry. I can not say how large is his pepsin secretion, but his hydrochloric acid output is great, as one's hands will tell if he dissects a shark's

stomach and handles its contents. Many sharks get their food as scavengers about city harbors, particularly if there are cattle-slaughtering establishments in the vicinity. Thus my happy hunting ground for sharks at Key West, Florida, was off Slaughterhouse Point. But even here the 10-foot tiger and requiem sharks cruised around as individuals and not in bands. However, sharks do apparently school up temporarily when feeding on fishes that go in shoals, and when preying on whales moored alongside vessels for "cutting in." But this schooling is for feeding only and is entirely temporary.

But *Rhineodon typus,* the largest of recent sharks, has no enemies, and being provided with backwardly hooked teeth only one eighth of an inch long, it is the enemy of no fish larger than a sardine. It feeds on small planktonic organisms, on crustaceans, squids and sardines as I have shown in a paper now in press. These statements in this introductory section run counter to the popular conception of a shark, and particularly of a large one. They call for proof and this will now be forthcoming.

For a considerable part of the data herein contained, I am indebted to the efficient services of the U. S. Hydrographic Office, and it is a pleasure to acknowledge that indebtedness.

## Rhineodon Associates with But Is Not Afraid of Sharks

Most sharks are more or less solitary, especially in their feeding habits. Only three kinds are known to me to go in schools—the whale shark, the basking shark (*Cetorhinus maximus*) and the nurse shark (*Ginglymostoma cirratum*). The first two grow to great size (30 to 40 feet) and the third to 8 or 10 feet. However all have small teeth and all are harmless.

On the other hand, almost all other sharks, especially large ones, are cannibalistic, freely attacking and feeding upon their nearest relatives. In another article (Gudger, 1932) I have brought together all my own observations of

sharks feeding on their own kind, and all the accounts in the literature known to me. However, the whale shark, because of its small teeth, feeds on no fish larger than a sardine as I have shown in an article in press.

That the whale shark sometimes associates with these ravenous cannibalistic sharks seems to me so extraordinary a matter as to be worthy of attention. The records of such associations are few but interesting as will now be seen.

Captain James Steuart (1862) wrote that off Ceylon "Sharks of the ordinary description are frequently seen: and on two occasions my attention has been called to spotted ones of such monstrous size as to make the common ones at their sides appear like pilot-fish." Next we hear of Rhineodon among other sharks in a book by Julian Thomas (1887). While at anchor in Redscar Bay, southeastern coast of New Guinea:

> A school of sharks twenty-five to forty feet long now surrounded us. . . . The [largest] fish came right underneath the bows, and then quietly floated astern on top of the water. . . . This was an enormous mottled brute, which seemed as long as our ship. . . . The sharks were all around. . . . The "boomer" appeared to lead them, and they swam around us both to port and starboard.

Then from another part of the world—the Gulf of Lower California in May, 1911—there has come an account rather similar to Steuart's. It is of a 10-foot shark which in May, 1911, played around a whale shark for some time, but the big shark paid no attention whatever to it. The whale shark being about 40 feet long, my informant wrote that the other seemed almost tiny beside it. Like this is the account sent me by Captain Tom Gifford, the fishing captain and guide well-known from Nova Scotia to Florida. Off Turtle Rocks, near Bimini in the Bahamas, he saw a 35-foot whale shark and two 8-foot common sharks swimming through a school of sardines. Neither shark paid any attention to the others.

Another but related kind of association is described by Max Weber (1902, 1913), who, in 1899, in the strait of Buton between the islands of Buton and Muna (Celebes

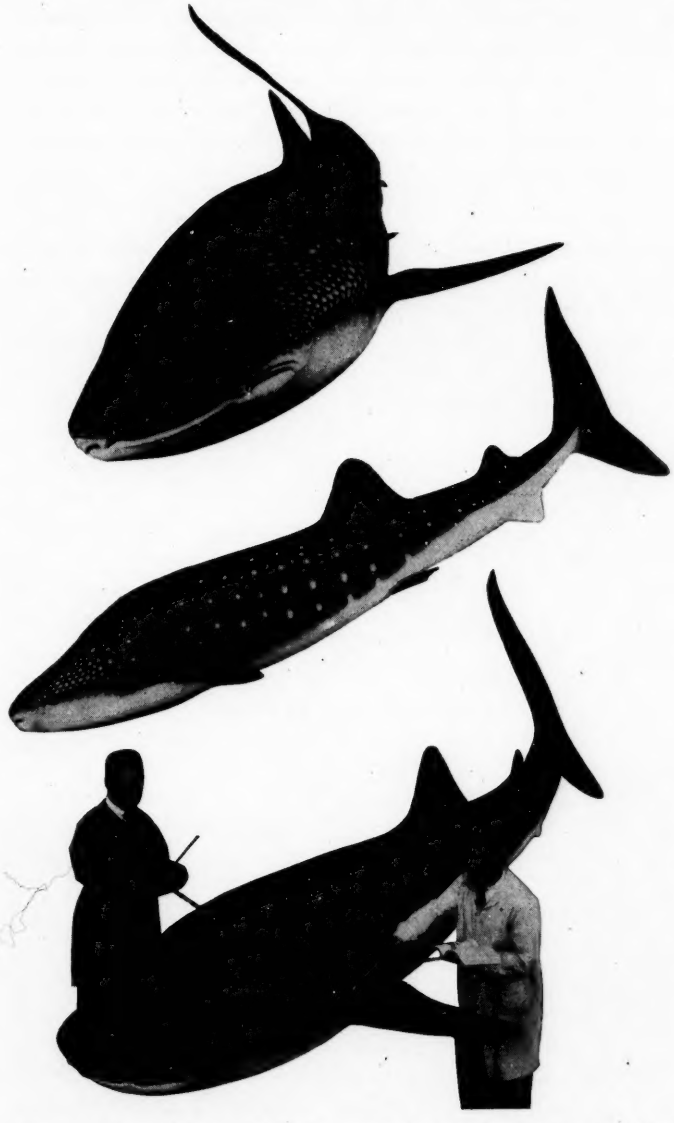

FIG. 1. Three views of the 18-foot mounted whale shark in the American Museum. Dr. James L. Clark, head of the Department of Preparation, and Mr. Ludwig Ferraglio, who mounted the skin, are shown restoring the spots and bars when the skin was tanned.

group), saw a band of whale sharks in peaceful association not with sharks but with gigantic rays—probably of the Manta group. This incident was also described by Frau Weber-van Bosse (1905). There was no antagonism whatever, but evidence of fearless companionship.

That the whale shark seems to be immune from attacks by other sharks is attested by the following facts. I have personally examined three skins of the fish and one specimen in the flesh and its skin after this was mounted. Then I have received copies of the pictures of every whale shark which I know to have ever been photographed. Neither skins nor photographs show evidence of maiming or mutilation as the results of attacks by other sharks. Furthermore, in all the literature of *Rhineodon typus*, known to me, there is but one reference to any semblance of mutilations on Rhineodon. Beebe (1938) says that off Cape San Lucas, Lower California, they could day after day recognize certain whale sharks because:

> One had the first dorsal scooped irregularly out behind, another had both fins notched at the tips, and one . . . had, as a recognizable brand, a two-inch hole straight through the tail fin near the tip.

This immunity may in part be due to the great size of Rhineodon. Even a 25-foot maneater (Carcharodon) might hesitate to attack a 30-foot Rhineodon—especially since the latter, in addition to its great length, is very large in its forward parts and has an enormous mouth extending across the full width of the anterior part of the head. Then an additional deterrent is found in the thick dense skin.

The injuries to the fins noted by Beebe seem undoubtedly due to attacks by other sharks. The fins of this armor-clad giant (especially when the shark is small) might be mutilated by the bite of another large and voracious shark. For the hole in the upper lobe of the tail fin, I can find but two explanations—a bullet wound, particularly if made by the discharge of buckshot from a shotgun at close range, or penetration by a harpoon. I have a record of a somewhat similar harpoon wound in a large swordfish.

Just here it is well to note that, so far as I know, sharks, even in schools, do not ordinarily attack each other unless one is wounded. Thus at the "cutting in" of a whale, sharks in a peaceful school will attack the whale. But if a shark is wounded by a whaleman's spade, his fellows deserting the whale will fall upon him and almost devour him before he can sink out of sight.

### The Whale Shark Unafraid of Man

It is perhaps going too far to say that most sharks are afraid of man, but, so far as my experience goes, they certainly seem to avoid him—popular ideas to the contrary not withstanding. Thus my harpooner at Key West once offered to jump overboard on a 12-foot tiger shark, alleging that the shark would be panic-stricken and would at once leave for deep water. So sure was I that he was right, that I would have let him do it, had I not so badly needed that particular shark for dissection. I have read of such a thing being done to the utter discomfiture of the shark.

That sharks, espeeially the larger predatory forms, will attack man, when ravenous with hunger, when wounded, when frightened or when cornered, is well attested by a constantly growing literature. Some 80 attacks off eastern and southeastern Australia alone are thoroughly corroborated by incontrovertible evidence. But for all this, those of us who have studied the behavior of sharks toward man are persuaded that lacking the conditions set out above, sharks ordinarily avoid man and even flee from him. That this is not true of the whale shark, the present section of this article will demonstrate.

That the whale shark has no fear of man or of his weapons will be shown by many witnesses, but first there will be considered certain erroneous allegations that it is dangerous to man.

### Rhineodon Alleged to Attack Man

The whale shark was first *seen* in 1816, swimming under a vessel at anchor in the Bay of Meriveles near Manila,

Philippine Islands. It must have been a huge specimen, for as it passed under the vessel it was thought that the ship had parted her chain and was drifting over a bed of coral with light and dark spots. Piddington did not publish his report on this fish until 1835. In this article he included a number of hearsay accounts of the alleged ferocity of the whale shark.

One fisherman reported that "it was supposed that it often attacked and destroyed small fishing boats which never reappeared after going out to fish." A pilot for those waters reported this instance of alleged voracity of another specimen that a man who was pushed overboard in the hurry to look at the monster was "instantly swallowed by it." Then Piddington stated that "The native fishermen of the Bay of Manila quite corroborate this account, and speak of the monster with great terror." Last of all he quoted an officer of an American ship of which the mate was out in a boat in Manila Bay in a squall, and on looking behind him he "saw the open jaws of a huge fish almost over him . . . he threw the boat's tiller into the mouth of the fish . . . the jaws closing with a tremendous crash . . . the fish . . . like a spotted whale . . . dived . . . and was seen no more."

So much for lively imaginations in unscientific observers under strong excitement. And, recalling my excitement when I saw a 31.5-foot whale shark in the flesh, I can understand this.

This excuse can not be offered for the next reported. In 1915, David Starr Jordan noted that he had had sent him from the island of Cebu in the Philippines a photograph of a Rhineodon which was alleged to have had in its stomach "7 leggings, 47 buttons, 3 leather belts and 9 shoes." Now Smith (1849) had written that the esophagus of a specimen dissected by him was small (apparently about four inches in diameter) with a right-angle bend which would prevent anything larger than a small fish going down it. I judge that the things listed by Jordan had come from the stomach of another cross-striped shark

in the Philippines—*Galeocerdo,* the tiger shark, in which the mouth grades into gullet and this into stomach without any material decrease in size. From the stomach of an American *Galeocerdo* I have taken such large objects as a horse's head and a cow's (dehorned) skull.

## Rhineodon Unaggressive toward and Unafraid of Man

From these few incredible reports of attacks on man by Rhineodon let us turn to the reports from many observers who have found the whale shark both unaggressive toward man and unafraid of him.

### In the Indian Ocean

Thus earliest of all, Smith (1849) says of the first specimen (16 feet, 6 inches long) ever studied, figured and described:

> At the time it was discovered [1828], it was swimming leisurely near the surface of the water [in Table Bay, off Capetown] with a certain portion of its back above it. When approached, it manifested no great degree of fear, and it was not before a harpoon was lodged in its body that it altered its course and quickened its pace.

Along the same line, Buist (1850) says that when the "Mhor" (the native name of Rhineodon) is harpooned off Kurrachee, northeast India, it is allowed to tire itself out before the boat is hauled alongside in order to kill it.

Wright (1870), who saw four specimens in the Seychelles Islands and photographed two, says that "it is a quiet, harmless fish . . . it never attacks or molests men, and while it reigns a monster among sharks, it is not . . . as formidable as the common dogfish." Just here, however, another quotation from Wright (1876) reads as follows: "Stories were told me of how a harpooned Rhineodon, having by a lightning-like dive, exhausted the supply of rope which had been accidentally fastened to the boat, dived deeper still, and so pulled pirogue and crew to the bottom." This, however, is not an offensive but a defensive reaction—the fish was simply trying to get away from its attackers and carried the boat with it.

IN THE EASTERN PACIFIC

In these waters, from the coast of Lower California to the Galapagos Islands, I have data on the entire fearlessness of the whale shark for man. But of these large numbers of accounts, I shall give only the more outstanding evidence. And interestingly enough this comes from one particular region.

Off Lower California, along the western coast of this peninsula, Rhineodon abounds and is so unafraid of boats and men that he might fairly be called the friend of man.

Zane Grey (1925) tells of running alongside a huge specimen off Cape San Lucas but the fish swam ponderously and stolidly along and paid no attention to them. Then they determined to try to catch it. Two futile attempts were made to gaff it in the tail. "The fish did not appear to be aware of our ambitions and evil attempts." At the second attempt, "The fish did not change speed or position." The third effort was successful. The fish put on speed and ran ahead dragging the boat but presently swam as before. At the first attempt to harpoon it, "The iron (*i.e.*, harpoon) came back bent." The second attack with the harpoon was more successful but after a sharp flurry the iron pulled out. Finally another harpoon was lodged and then the great shark, annoyed by all these prickings, dived and at 1600 feet the hook and harpoon pulled out and the fish went about its business. At no time did the fish take any offensive attitude, it merely sought to get away from its annoyers.

Beebe (1938), in the same general locality had a very similar experience. For hours his boat ran alongside a huge Rhineodon. Two of his men with a large harpoon held between them leaped from the deck of the *Zaca* onto the back of the fish and drove the harpoon home. The whale shark dived and presently reappeared some distance away. The boat ran close and two more attempts at harpooning were tried, but the shark had "set" the muscles of its back and the harpoons came back bent and

blunted "as if they had struck steel." Finally in endeavoring to bring yacht and fish together, when the fish felt the pull, ". . . he tore out the harpoon as if it had been a pin and was off, without any undue haste, for the open Pacific." Then Beebe adds "They never attack, but are troublesome when by accident they get entangled in a fish net."

A strong man may drive a sharp fine-pointed harpoon *into* a whale shark's skin if the point strikes at a right angle to it. I doubt if he could drive it *through* it. The nurse shark of Southern Florida has a hide less than an inch thick, yet, when the shark "sets its muscles" and tautens its skin, it is hard to drive a harpoon into it. Time after time, I have seen a harpoon, thrown by a strong man, rebound from the fish's back. When the whale shark is fired at with buckshot, these simply rebound. So also will shots from a high-powered rifle, unless the shots strike the skin squarely—*i.e.*, perpendicularly. Clad in this well-nigh impervious armor, Rhineodon fears not man.

I have a secondhand account of a tuna-fishing captain off Lower California who landed on and stood for some time on the broad back of a whale shark. I have not been able to verify this account and so quote it for what it may be worth. Personally I think it not impossible and even not improbable that such a thing might be done. With its thick hide, the great shark would hardly know that the man was standing on his back.

Since writing the above, I have received an attested account of a man landing on and walking about on the back of a whale shark. The shark paid no attention whatever to the man.

### IN THE WEST-CENTRAL ATLANTIC

In the Florida Keys a considerable number of whale sharks have been seen and three or four have been captured. In all these their behavior toward man has been

of a kind. One only of these captures will be referred to. This account is of that specimen which started me on a long-time study of Rhineodon.

Concerning the 38-foot whale shark captured at Knight's Key, Florida, in May, 1912, my informant wrote (Gudger, 1915, p. 352):

> I was surprised that the fish did not put up any fight [as an ordinary tiger shark does]. He proved to be a sluggish monster, and seemed to fail to realize that anything particular was happening to him. He swam around slowly in circles, dragging the boats around after him.

A score of similar solitary incidents from various seas might be noted, but it does not seem necessary to give them. From now on the cases cited will be those which, out of the ordinary run, will add new and unusual evidence of the unafraidness of the whale shark.

In the Gulf Stream off Havana, the whale shark is not very infrequent, and, since several have been taken, it is pretty well known. Two of the fishes offered little resistance, and the second showed such extraordinary docility that the story of its capture must be carefully narrated.

The skin of the first shark was mounted for display and brought revenue to its captors, but not being tanned it presently went to pieces in the heat and dampness of summer. When the second was seen, the fishermen and their backer determined to try to capture it for the same purpose. The data for both captures were reported to me by my indefatigable correspondent, Dr. W. H. Hoffmann of the Laboratorio Finlay, Havana. Dr. Hoffmann personally investigated both captures and saw the two fish. His data were accompanied by photographs of the sharks.

The first fish allowed the boat to be "nosed up" against it and even after being harpooned did nothing but try to get away. The second Rhineodon manifested such incredible docility that had not Dr. Hoffmann seen the lasso about the body, even he would not have believed it. I have no such account of unafraidness of Rhineodon reported in my 27 years' study of the whale shark. It sounds like a large "fish story."

This or another or a succession of whale sharks had been seen for three years off Cojimar, a fishing village about five miles east of Havana and had been named "El Elefante." This or another whale shark was captured in 1930. The fish lay inert at the surface of the water and allowed two launches to approach it rather closely on either side. Then the fishermen by means of long bamboo poles so manoeuvred a looped thin steel cable as to get it over the head of the shark in such fashion that the cable encircled the body in front of the right and behind the left pectoral fin. Thus, when the cable was drawn tight, it was impossible for the fish to get free of it. This capture took place about a mile from shore in water fairly deep, and, to prevent the escape of the fish by diving, the fishermen fastened to the cable two empty steel petroleum drums. Thus secured, the fish, which up to this time had offered little resistance, was harpooned and fired at with a rifle. Finally, between being towed and driven, it was brought into shallow water. Here, by reason of wounds in the gill region it finally bled to death some 24 hours after being lassoed.

This is to me the most amazing case on record of the lack of fear of man by the whale shark, and of its indifference to what was happening to it, coupled with a total absence of defensive endeavors.

This stolidity or fearlessness has led the Cubans, who seem to know this great fish pretty well, to call it "pez dama." This may be translated "checkerboard fish," because the longitudinal ridges on its sides and the vertical yellow or whitish bars make checkerboard-like squares in each of which is a yellow spot. But with a fine knowledge of its psychology, the Cubans also translate "pez dama" into "lady fish" or the "gentle one"—terms which, as the accounts above show, are surely applicable.

### The Whale Shark Unafraid of Ships

Since man can only approach Rhineodon by means of boats, accounts in the preceding section of its unafraid-

ness of man have also included a fearlessness of boats. However, its unafraidness of man has been the dominant matter in that section. In this section it is the fearlessness of boats rather than of man that will predominate. An effort will be made to avoid repetitions, and the endeavor will be to show that the whale shark does not avoid boats and ships, but in some cases actually seeks them. This is in contradiction to the fact that ordinarily sharks avoid vessels.

The ordinary shark either avoids ships or "hangs around" them waiting for scraps from the galley for food. Rhineodon does neither. As I have shown in another paper (awaiting publication) the whale shark is a surface feeder, and his food consists mainly of small floating and swimming organisms. His largest articles of food seem to consist of sardines. That our fish not only does not avoid ships but seems to seek them (especially fishing vessels) will now be shown.

### IN THE INDIAN OCEAN

That Rhineodon does not avoid small boats even with men in them is an inference to be drawn from the accounts given above, and is plainly evidenced by the last one in the preceding section. That it has what is almost a liking for boats, large or small, is attested by one of our earliest authorities. Wright (1870) says that in the Seychelles in his day ". . . it now and then rubs itself against a large pirogue, as a consequence upsetting it, but under such circumstances it never attacks or molests the men." It may be conjectured that this is from playfulness, or that it is a manifestation of amiability such as is displayed by a friendly house cat.

Some more or less incidental notices will now be quoted. Thus Foley, as early as 1835, wrote that in the Bay of Bengal an animal as large as a whale but "spotted like a leopard . . . came close under the stern of the ship," so close that they threw large stones down on it but of which it took no heed.

IN THE WESTERN PACIFIC

Now come two isolated but very interesting accounts from the extreme western part of the Pacific of the absolute unafraidness of the whale shark to ships.

*In Redscar Bay, Gulf of Papua.* Julian Thomas (1887) says that, here on the southeastern part of New Guinea, a great shark—the "boomer"—"an enormous mottled brute [at least 40 feet long] . . . came right underneath the bows and then floated quietly astern on the top of the water. We could have touched him with our hands by leaning over the bulwarks." Then the great shark calmly swam all around the vessel as if inspecting it.

*In Buton Strait, Celebes Group.*—Max Weber (1902 and 1913) writes that in this strait between Buton and Muna Islands, there played around the *Siboga* a school of whale sharks and great rays (probably mantas). They seemed almost on a tour of inspection—very like Thomas's "boomer." Frau Weber-van Bosse (1905) reported a detail of some interest. One "for a while drifted around the ship in very close proximity. It must have struck the ship, for its head had rubbed some of the red paint off our ship"—a happening which we shall later hear of again on the other side of the Pacific.

IN THE WEST-CENTRAL ATLANTIC

Attention will now be called to a series of accounts of cases of unusual familiarity of whale sharks for vessels in the Caribbean Sea, Gulf of Mexico and contiguous waters.

*In the Caribbean Sea.*—The first account pertains to an unusual behavior of a Rhineodon around a vessel in the harbor of St. Marc, at the western end of Haiti. There the great fish "hung around" a steamer while she was being loaded with bananas at night under strong electric lights. Boys in small boats were so frightened that they boarded the ship letting their skiffs drift away. On one round, the whale shark came up under the companionway, and, raising its head, it dislodged the platform at the bottom of the ladder. It stayed around the ship for about

two hours and occasionally bumped against it, the impact being noticeable.

*In the Bahama Islands.*—In these waters, Mr. E. R. Fenimore Johnson in his ketch *Elsie Fenimore* ran into a school of tame feeding whale sharks which swam around his vessel so closely that he made a motion picture film of them. A copy of this film has been kindly presented to the American Museum. Some of these sharks came so close to the *Elsie* that "we could have jumped astride their backs." This parallels the experiences of Grey and Beebe off Cape San Lucas, Lower California, previously related.

*In the Gulf of Mexico.*—For this region the reports are from ships making passages. From one ship a school of six whale sharks was seen. "They were entirely unafraid, one left the school, came alongside the ship, and then went back to the group." From another ship, "The (12) fish approached close on the port bow, and the nearest had to steer off to clear the ship, passing leisurely at a distance of about 40 feet." The third report is of 30 fish "not disturbed by the ship's passing at . . . 20 feet from the nearest but they swam lazily along." From another vessel, six were seen so close at hand that the vessel's wash broke over them without disturbing them. From another ship comes a report of four in pairs that were so sluggish that they made no effort to get away from the steamer.—It must be emphasized that these vessels were not at anchor but each was "making a passage" at 12 to 15 knots per hour, and the wonder is that some of these great sharks were not struck by steamers.

### IN THE EASTERN PACIFIC

From the Atlantic, let us now go to the Pacific Ocean in which I will record the most amazing cases of whale-shark-ship affinity ever put upon paper. And first of all is a case almost a complete parallel to that noted in the harbor of St. Marc, Haiti, Caribbean Sea.

*In the Hawaiian Islands.*—The incident now to be re-

lated took place at Kukuihaele landing on the north coast of Hawaii in September, 1938. The ship *Mapele* was at anchor, discharging cargo, when a whale shark appeared swimming around the ship amid the debris thrown overboard from the galley but not feeding on this. Then "It swam leisurely around for about 20 minutes, bumping into the mooring lines a number of times. Once, as it was swimming over one of the lines to a buoy, the ship lifted, throwing the shark partly out of water. Then it swam off slowly."

*Off the Coast of Lower California.*—In this locality whale sharks are very abundant, very tame and even very amiable. This has been generally known for a number of years, but I am now able to give some very definite data in addition to that from Zane Grey (1925) and Wm. Beebe (1938) quoted above in the section on unafraidness of man.

First of all in 1926, Mr. Keith Spalding, a fellow and patron of the American Museum, wrote me that, on a fishing trip to Cape San Lucas, he and his company daily associated with whale sharks—shark and boat moving along side by side. Of one fish he wrote that in the passage between Ceralbo Island and the mainland his launch ran for several hundred yards alongside a whale shark only 15 or 20 feet away. "It submerged and swam under the boat as though looking us over." Then in that same summer, Mr. Mack Sennett, the motion picture producer, while on a fishing trip in the same region, had with him a submersible movie camera with which he made underwater moving pictures of Rhineodon at close range. Mr. Sennett has generously presented a copy of this film to the museum. Examination of the film corroborates Mr. Sennett's statement that at no time did any whale shark show any fear or resentment, or offer any violence whatever to the boat.

There are now to be presented some amazing data on the utter fearlessness of Rhineodon with regard to tuna-fishing boats in this region. These accounts have so far

as possible been verified. Moreover each account came to me without knowledge that similar accounts had been sent in by other men. As will be seen all accounts corroborate each other, all dovetail together.

Tuna-fishing vessels are of fairly deep draft aft, but are much cut away forward. Such a vessel bumped a whale shark in 1932, and the shark was then seen making off with a smear of red copper paint across its back. This recalls what Frau Weber-van Bosse wrote (1905) of the whale shark seen in the strait between Buton and Muna, Celebes Group. This had on its back a smear of red paint which she thought had come from the ship's bottom.

In the tuna-fishing to which reference will now be made, "racks" will be referred to. These are grate-like metal frames slung over the stern of the tuna boats and aft alongside the stern on each side. Thus the hinder part of the boat is enclosed with these racks. On these the fishermen stand and, with stout rods and strong lines ending in barbless feathered hooks, haul in the tuna.

It has been known for some time that whale sharks are generally found associated with tunas in this and other regions. However, just here we are particularly interested in the behavior of the sharks toward the fishing vessels.

Thus Captain J. M. O'Neil of the tuna fisherman *Pacific Queen* out of San Diego, when fishing for tuna on Gorda Bank off Cape San Lucas on April 9–14, 1939, had a most interesting experience. He writes:

> Each day at least one whale shark was circling around us, under us and everywhere but over us. In going under our stern, its large dorsal fin would strike on the fishing rack before it went completely under water and the upper part of the tail fin would do the same. Twice while in the fishing rack I had one of these big fish come along under me. . . . I reached down and put the end of my fishing pole against his back . . . and it was as hard and unyielding as a brick wall. . . . We became so accustomed to them cruising around us that they did not cause any interest after the first day or two. They are inoffensive in the extreme.

Captain O'Neil describes another experience when fishing off Cape San Lazaro, Lower California, on July 16

and 17, 1939. Each day a whale shark was seen and each swam about the vessel and showed no fear of it. Then he concludes:

> In appearance and action they were exactly as described in my earlier report—very amiable, slow and have an inclination to sound and scratch their backs on our keel.

Last of these accounts of the amiable whale shark is found in a clipping from the San Pedro, California, *News Pilot,* kindly sent me by Mr. R. S. Croker, editor of *California Fish and Game.* The tuna-fisher *Magellan,* while out a few days earlier on Thetis and Uncle Sam Banks off Lower California, saw numerous whale sharks. Some of these "remained about the boat for hours, now and then rubbing against the boat as if eager to rid themselves of vermin of the sea."

And so this section ends even as it began—with the unafraid Rhineodon using the fisherman's boats as back scratchers—off the coast of Lower California in 1939 and off the Seychelles in the western Indian Ocean, seventy years ago.

### Some Unfortunate Results of This Unafraidness

Before closing this paper, brief reference must be made to an unfortunate outcome of the unafraidness of Rhineodon and of what might not inappropriately be called his predilection for ships large and small. As some of my readers have already conjectured, this habit of our shark leads to his own undoing—and he is sometimes "bumped" by small vessels and sometimes impaled on the bow of a larger one. In another paper (Gudger, 1940), I have brought together three accounts of whale sharks "bumped" by small vessels. Then there were 12 definite accounts of whale sharks rammed by ocean steamers. Illustrating these accidents were seven photographs and one drawing. These rammings were all brought about by the sluggishness and unafraidness, by the "amiability" (to quote one ship captain) of the greatest fish in the seas, the Whale Shark, *Rhineodon typus.*

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# ON THE OCCURRENCE IN THE GULF COAST WATERS OF THE UNITED STATES OF THE TRIPLE TAIL, *LOBOTES SURINAMENSIS,* WITH NOTES ON ITS NATURAL HISTORY

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SOME months ago, in an issue of THE AMERICAN NATURALIST, I chanced upon a paper by Dr. E. W. Gudger, of the American Museum of Natural History, dealing with some notes on the occurrences and habits of the triple tail, *Lobotes surinamensis* Bloch, in our waters. Interested, I wrote to Dr. Gudger in regard to data I possess, and, encouraged by him, have since prepared this note, based on a rather large correspondence with various sources on the east Florida and Gulf coasts, and on various notes of my own made on the Texas coast. Besides those gentlemen mentioned in the text, I am particularly indebted to Ronald Halgrim, of Fort Myers, Fla.; to Stewart Springer, of the Bass Biological Laboratory, Englewood, Fla.; to H. B. Kraft, Stuart, Fla.; to Anthony Ragusin, of Biloxi, Miss., and to Mrs. Lyle McCaleb, of Aransas Pass, Texas, as well as to various others too numerous to mention, all of whom very kindly aided me in gathering information on this species.

Prior to the publication of Dr. Gudger's paper (1931), the recorded occurrences of this fish in any numbers on the Atlantic coast, from North Carolina to Georgia, were few, and none of these was from any farther south on the Florida east coast than Jacksonville. Later (1937) Hughes recorded it from Fort Pierce, Daytona and Canaveral Harbor, Fla. Now from Stuart and West Palm Beach, Fla., I have guides, issued for fishermen, in which the triple tail is listed among the other common game fishes of those localities. Further, Ernest Lyons, sporting editor of the *Stuart Daily News,* says, "We catch

them here in the St. Lucie and Indian Rivers occasionally during the spring and summer months."

### KEY WEST AND THE WEST FLORIDA COAST

At Key West, Lobotes is rare. Schroeder (1924) states that although it is occasionally seen in the markets, a twenty-pound specimen, which he saw in 1919, was considered an oddity by local fishermen, who had no name for it. This scarcity extends to the Tortugas also, for Gudger (*op. cit.*) says that in four summers' residence at the Tortugas Laboratory of the Carnegie Institution, he never saw one, and that the late Professor W. H. Longley, in fifteen years at the same station, had seen but three or four immature specimens.

On the west coast, at Everglades, D. Graham Copeland, reports that the triple tail is unknown.

Fort Myers and Punta Gorda furnish the first occurrence of the fish in sufficient numbers to attract the attention of sportsmen and commercial fishermen, and it is reported as an occasional habitant of both bay and Gulf waters in these localities.

At Bradenton, Englewood and Sarasota it is also taken in small numbers, but it is not plentiful in any of these places, and an experienced fisherman may go for three or four seasons without seeing a specimen. Tampa, at some seasons of the year, has fairly large numbers present, generally over rocky bottom in the main ship channel, but at Tarpon Springs, under the name of "sunfish," it is accounted a rare and unusual visitor. From this point north to St. Mark's, Fla., I have no knowledge of its occurrence.

St. Mark's, however, reports one of the largest catches of which I have any record. An Apalachicola snapper boat, operating at St. Mark's Rock Reef in 1921–22, took six hundred pounds from a school on one trip and two thousand pounds on another.

At Pensacola, Francis W. Taylor, of the Warren Fish Company, says, "On numerous occasions in the past,

fishermen have brought us specimens of *Lobotes surinamensis*. It is the writer's understanding that during the summer months these fish can be found in the salt water lagoons around Pensacola. I don't intend to convey from the above, however, that the fish are plentiful, for several seasons may pass without us getting a single specimen."

### Alabama, Mississippi and Louisiana

Bon Secour, Weeks and Mobile Bays, in southern Alabama, are favorite haunts of the species. J. W. Sandlin, of Magnolia Springs, writes that it is not unusual for an experienced fisherman to take from sixty to ninety pounds per day. Barney Sheridan, of Mobile, says, "It is our understanding that 'blackfish' [a local name] are caught in great numbers in the waters of Mobile Bay during the summer." This is confirmed by Carlton Thomas, sporting editor of the *Mobile Times*.

Along the Mississippi coast to the westward, this abundance continues. Pascagoula, Biloxi, Gulfport and Bay St. Louis are all reported as having extensive tripletail populations during the summer months. Men familiar with this section of the coast say that the fish is probably as thickly concentrated here as anywhere along the adjacent coasts, although some seasons it is very rare. Fair numbers are taken by both sportsmen and commercial fishermen, generally with hook and line, but also, upon occasion, with trammel nets and seines.

Louisiana appearances have been summarized by Gudger (*op. cit.*) who says that Lobotes "occurs out in the Gulf, and comes inshore only in the passes at the mouth of the Mississippi River, and in the inlets between the outlying coastal islands, and around these islands." Gowanloch (1933) gave the figure of a specimen taken at Quatre Bayou Pass. Gunter (1935) recorded one specimen taken in Barataria Bay during September, 1933. However, during several years fishing in and around Bay Terrebonne, I never saw a specimen, and between this

point and Sabine Pass, Texas, there is no recorded occurrence (so far as I know), nor has it been possible to develop any information as to the presence of the triple tail in these waters.

### In Texas Waters

Port Arthur reports the "rockfish," as it is known colloquially, present in small numbers, both at the Sabine Pass Light and at the outer Sabine bank, about forty miles offshore. At Galveston, in the early spring, it frequents the shores of the inside bays; later in the season it moves to deeper water about the jetties at the harbor mouth. It is also found around the hulk of an old concrete ship, which lies to the east of the main channel, and is quite plentiful at times about the Bolivar Slips.

Freeport and the coast west of there to the mouth of the St. Bernard River afford the greatest concentration of the species known to me in Texas waters. It is taken off the jetties at the old mouth of the Brazos River, at Freeport, by hook-and-line fishermen, and occasionally large hauls are made over the sand flats at the river mouth, as many as sixteen hundred pounds being taken in two hauls of a three-hundred foot seine at this place. At the new mouth of the Brazos, a few miles west of Freeport, large numbers were taken in 1937 by haul seiners, in water that was red with silt from the flooded river. Between this point and the mouth of the St. Bernard, still farther to the west, surf fishermen occasionally catch a triple tail while fishing for "reds" (*Sciaenops ocellatus*). They are also caught in the St. Bernard itself, one of twenty-seven pounds being taken four miles above its mouth in the season of 1935 or 1936.

At Rockport and Aransas Pass, and in Nueces Bay, they are present, although apparently not in large numbers, and they are occasionally present at both Port Isabel and Brownsville, though infrequently.

From the foregoing it would appear that, while present in some numbers on our eastern Atlantic coast as far

south as West Palm Beach, Fla., it is exceedingly rare about the southern portion of the Florida peninsula, including the keys, and is apparently absent on the west coast until Fort Myers is reached. From this point on it increases in numbers as we follow the Gulf coast, reaching its greatest concentration, although spottily, along the northern shores of the Gulf of Mexico from St. Mark's, Fla., to the mouth of the St. Bernard River, in Texas. It may often be absent from its former haunts for several seasons, only to reappear in about the same numbers.

## Natural History

The season of occurrence on the Gulf coast generally runs from April to early October, varying somewhat with the temperature, warm weather encouraging a longer stay than common, if it occurs late in the season.

*Habitat.* The habitat of the fish, as known and reported, exhibits some curious inconsistencies. Ordinarily it seems to prefer the mouths of rivers, passes and bays opening into the Gulf, even displaying a good deal of indifference as to the degree of salinity of the water. In such places it is apt to be found in water of from one to three fathoms in depth, congregating around wrecks (Kelly, 1923), buoys, boats, beacons, piling, flotsam, rock jetties and even, in some instances, close to shore under the shelter of fallen tree tops and brush (Gudger, *op. cit.*). This does not always hold true, however, as in the spring and summer, especially at night, they may approach quite close to shore, over shallow sand flats, and at such times may be taken in nets.

Further than this, and at complete variance with the foregoing, is the fact that it may at times be taken over rocky or coral bottom, such as the snapper banks, in depths up to fifteen fathoms or more. This is so reported from several points in both Florida and Texas, and from the Campeche Banks, near the cost of Yucatan, adults and young (the latter from the stomachs of groupers) have been reported from a depth of twenty-five fathoms.

G. B. Skelton, of Houston, says that he has seen them in the Gulf one hundred miles offshore, and one fish was reported as having been taken sixty-three miles southwest of Cape San Blas Light, Fla.

*Size.* Triple tails up to forty-one pounds have been reported, but the average seems to be between two and fifteen pounds, with a preponderance of seven- to ten-pound specimens. Occasionally larger fish are taken. Gowanloch (*op. cit.*) figures one of thirty pounds' weight, while I have reports of 14, 16, 17½, 18, 24, 27, 36 and 41 pounds, all scale weights, not guess work. The last was taken by F. B. Brogniez, of Houston, near the old concrete boat in Galveston harbor. Unfortunately, it was impossible to gain any accurate data on the length or girth of these fish. Breder (1929) reports that the fish may reach three feet in length, and the probability is that such a specimen would weigh from thirty-five to forty pounds. Gudger (*op. cit.*) lists one of thirty-five and one-half inches standard length, which would have measured about forty-two inches overall.

Small specimens have been reported from inland bays and brackish water to the open sea. (Note observations on Campeche Banks.) Evermann and Marsh (1902) mention two examples, one of six and one-half inches, and another of eight inches, from Puerto Rico; Schroeder (1924) mentions a six-inch specimen from Key West. Beebe and Tee Van (1933) say that small specimens are taken among the small sargassum weed in Bermuda; Meek and Hildebrand (1925) list a seven and one-quarter inch specimen from Colon, Panama. The smallest known to me is one of less than three and one-half inches, taken in the boat harbor, Rockport, Texas, in Aransas Bay, in August, 1939. This is now in the possession of Gordon Gunter, marine biologist, Texas Game, Fish and Oyster Commission at Rockport. Mr. Gunter says that similar individuals are reported to be seen there occasionally among the algae growths during the summers.

*Color.* A wide variation in color seems to be character-

istic, especially among the smaller members of the family, the fish varying from yellow, yellow and tan, to yellow and black, bronze and blackish silver. Gudger (*op. cit.*), working from a very complete series of small specimens, describes the coloration for juvenile or small fish as follows:

> The body is brownish or greenish—darker at the back and shading off to lighter downward—with yellowish or greyish blotches. The fins are generally of the same color as the body, except the pectorals, which are whitish translucent. The caudal has a variable broad marginal band of the same color as the pectorals, especially marked in the young. The dorsal and anal fins have the general body color, but are narrowly margined with lighter. Two narrow streaks on top of head between the eyes extend to nape. One streak runs from posterior margin of eye upward to nape, and another one runs from posterior margin of eye downward to the lower angle of the preopercle. These streaks are not very sharply defined. In very small specimens the brownish pigment tends to become concentrated in the centers of the scales and in certain areas, forming ill-defined cross bars and narrow longitudinal streaks along the rows of scales. There are frequently several dark spots on base of soft dorsal and anal, the latter sometimes distinctly ocellate.

Hildebrand and Schroeder (1928) say "young fish are sometimes marked with yellow and brown. A specimen observed at Key West had the yellow and brown colors of an autumn leaf." In some instances they are identical in color with the Gulf weed. Larger specimens agree in general with the description given by Jordan and Evermann (1896).

*Shade seeking.* Dr. Gudger, in the note previously referred to, has commented on one habit of the triple tail which is unusual, namely, their proclivity for seeking the shelter of wrecks, piling, buoys and beacons, where, canted at an angle of forty-five degrees from the perpendicular, the fish lies in the shade, possibly sleeping, certainly lethargic. Hughes (1937) remarks that they are often taken from under shrimp boats anchored several hundred yards offshore. He says, "Often when a boat has been anchored only a few hours it is possible to take from ten to twenty-five triple tails from under and around it."

Carlton Thomas gives a very interesting commentary on this habit when, in speaking of the fishing in lower Mobile Bay, he says that it is found

usually near the mouth of the bay in the shadows of beacons or channel markers. Sometimes they are caught in open water, but fishermen always seek them near the beacons, where they lie on top of the water and are easy to spot. They are always in the shadow. I have never seen them in the sun. The usual method of fishing for them is to use a power boat and go from beacon to beacon, watching the shadow cast by the (beacon) house. They are usually found on their sides and are not easily disturbed.

A number of my correspondents have noticed this lethargic state, one saying that while in this condition small specimens might be taken with the hands, and another that they are taken with dip nets. Mr. Gunter tells me that small specimens at Rockport, Texas, float slowly and lazily, but always just out of reach of the hands. This would indicate that the fish is not necessarily in a somnolent state when floating in this manner. All concur in the statement that the fish is very active and timid when aroused.

*Names.* This habit has given rise to a number of names, all of them descriptive, among which may be listed (besides those already given) "snag-drifter," "dormeur" and "sunfish." One listed by Gill (1856), *i.e.*, "flasher," I have never seen any satisfactory reason for, but Mr. Gunter suggested that it comes from the action of the fish when aroused from the lethargic state referred to above. At such times as it rights itself, it does so with a swirl, flipping quickly to an upright position, and it may be that the glint from its side at such times earned the name from fishermen accustomed to its capture. The appellation of triple tail is also a descriptive one, for the deeply lobed dorsal and anal fins combine with the caudal to give the appearance of three tails, and to form the basis for the name.

*Schooling.* They school at times. Gudger (*op. cit.*) notes this characteristic habit, and besides those instances at St. Mark's Reef and Freeport which have already been mentioned in this note, Wallace Caswell, of Panama City, Fla., says that he has seen small ones in schools, some distance offshore, apparently migrating. Size of the fish in a school does not seem to be uniform, as in one haul of a seine at Freeport, Texas, the fish ran all the way from

two or three pounds in weight up to thirty-four. The majority ran from five to fifteen pounds, with only an occasional larger specimen.

*Spawning.* Spawn has been reported in three cases. In Florida a fish with white roe was reported in midsummer. In Mississippi, roe is present at the time of their occurrence off Biloxi, the eggs being largest between July and August, and smaller, or absent, later on. Fish at Freeport, Texas, have spawn in them during the last few days in June, and in July and August, but after that the roe is slight or absent.

*Its Food.* This, as indicated by bait preferences, is varied. Live sardines(?), crabs and cut bait are used on various portions of the coast, and, occasionally, either fresh or salt meat. During the early spring they are reported to frequent the beaches, where they feed on spawning crabs. It is also said to feed on young barnacles found on floating drift. It will strike at artificial bait, though rarely.

*Sought by Anglers.* A bold biter, and strong and enduring in battle, the triple tail leaves little to be desired by the sportsman. Some years ago, at Freeport, Texas, I watched a commercial fisherman catch two of about seven or eight pounds apiece. His bait was shrimp, and he was fishing about four feet deep, using an enormous cork. When the fish struck, they did so so sharply that the cork went under with a distinct plop. Neither made any effort to jump, but both put up a hard, boring, smashing fight, bending the heavy cane pole almost double. This type of fight seems to be characteristic of the species, and although Kelly (*op. cit.*) states that they jump when hooked, only two of my correspondents have seen them do so. One very curious trait is mentioned by Louis Gorenflo, of Biloxi, Miss., who says,

> They will put up a good fight if hooked as soon as bait is taken. If allowed to swallow bait, like any other fish it can not fight long with hook deep down in gullet. [This may explain why some do not jump.] *If pulled slowly, can sometimes be brought right into the boat like a crab, that is if the hook is not set.*

*Use as Food.* Despite the fact that the extreme toughness of its skin and scales make it a very difficult fish to prepare for the table, the triple tail is unquestionably one of the finest of our salt-water food fishes. Every one who has ever had any experience with it rates it well above the average, especially when baked.

Curiously enough, the fish markets are in many instances loath to handle it. Mr. Gorenflo states that at Biloxi they do not care for it and will give only six to eight cents a pound for this fish, with a preference for those of four to seven pounds. At Freeport, Texas, seiners do not like this species, for there is little or no market for it, most people being unfamiliar with the fish and chary of trying it. One thing that militates against its becoming popular is the fact that rarely does a sufficient supply reach the markets at any time to make it worth while to handle it. The great majority are taken on hook and line and are consumed by the fishermen who take them. Consequently, the general public has never had any opportunity to acquire a taste for triple tail.

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# THE MUTATIONAL PROCESS IN DROSOPHILA MELANOGASTER UNDER AVITAMINOUS $B_2$ CONDITIONS

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THE present investigation was undertaken with the purpose of studying how far the mutational process is dependent upon developmental conditions. The question is: whether such a dependence exists, whether the rate of the so-called spontaneous mutational process is affected by the conditions that in an individual determine its developmental possibilities as well as its physiological state?

At present the experimental study of this problem (apart from the Lamarkists experiments) is in its initial stage and for this purpose vegetal objects only have been used so far (Schlösser, Döring). It is to be noted that the problem to be solved excluded the possibility of applying factors that directly influence the germ cells (such as temperature, strong chemical influences).

## ASEPTIC CULTURES AND THE NUTRITIVE MEDIUM

Among the conditions that are of decisive importance in the development of an individual but do not directly affect the germ cells, the nutritive régime is of prime importance. As the Drosophila feeds mainly on fermentative microorganisms an experimental control of the flies' nutritive régime is possible in aseptic cultures only. Such Drosophila cultures were first obtained by Guyénot as early as 1911, at the time, that is, when the chromosomal theory of heredity was just beginning to develop. Guyénot indicated the importance of the aseptic cultures method for detecting the rôle of the ambiant medium with regard to the variability phenomena, but genetics did not make of it a current method of investigation.

The present investigation was begun in 1936, the experiments were carried out on the white strain obtained

from the Institute of Genetics of the Academy of Science, U.S.S.R. By means of frequent transfers (from 1 to 2 daily) a complete elimination of the microflora was achieved and in two months an aseptic white strain was obtained. The writer rears his aseptic culture in tubes containing an autoclaved ordinary yeast medium. This medium, including dead yeast cells, is quite appropriate as a food for the larvae and imagines. In transfers sterile glassware (Petri dishes for binocular control of the flies) and forceps were used. The aseptic conditions of the cultures were specially controlled by periodical inoculations made from the nutrient medium and the contents of the flies intestine.

In order to reproduce in the experiments as exactly as possible the conditions of the Drosophila's habitat the writer tried to breed the aseptic white strain on beer-wort-agar inoculated with *Bacterium pasteurianum*. In the localities where acetic acid is produced *Drosophila melanogaster* are very numerous (Henneberg, 1902). Sometimes this species is even called the acetic acid fly. Beer-wort-agar inoculated with *Bacterium pasteurianum* proved to be a medium that fully answered our purpose. On this medium the females lay as many eggs as on a medium inoculated with yeast (*Saccharomyces ellipsoides*) much more than in sterile tubes, that is on beer-wort-agar free of any microorganisms (Table I)[1] This circumstance is of great technical importance as it renders unnecessary the most tedious procedure of collecting eggs (under aseptic conditions).

The hatched larvae have a very slow growth and the great majority of them die without reaching the pupal stage, but the larvae themselves live long. Whereas in control cultures on a sterile yeast medium, or on beer-wort-agar inoculated with yeast, the pupal stage sets in on the fifth to sixth day; many of the larvae on a medium inoculated with *Bacterium pasteurianum* live as long as

[1] The Tables I to VIII inclusive have been omitted due to cost. They will be on file for some time and open to inspection.

from 18 to 22 days. The 18 to 22 days' old larvae are completely transparent and are but slightly mobile. The same picture may be observed in tubes with beer-wort-agar free of any microorganisms. The retardation in development may be due either to the *Bacterium pasteurianum* possessing or giving off some substances that prevent the setting in of the pupal stage (this theory is scarcely in accord with the data obtained for sterile tubes) or its cause lies in that the food given to the larvae is lacking in some substance essential for the normal course of development. The decision fell out in favor of the second theory, as proved by the experiment in which aseptic females were made to lay their eggs on a medium inoculated with a mixture of yeast and acetic acid bacteria. In this medium the larvae developed quite normally, not worse than in the control cultures, and the flies began to emerge on the tenth day.

It is natural to infer that the food of experimental larvae is lacking in vitamins of the B group, which are contained in yeast. Conclusive proof that vitamins of group B are essential for the development of Drosophila has been given by Bacot and Harden, Northrop, Baumberger, and it has recently been demonstrated by Vant t'Hoog that Drosophila larvae require the vitamins $B_1$ and $B_2$. By means of appropriate experiments we found that an addition of vitamin $B_1$ to the nutritive medium has no marked effect: the larvae die 18 to 20 days old without having reached the pupal stage. But tubes into which vitamin $B_2$ had been added (2 v per 5 cm of nutritive medium) are not behind the controls. In them the pupal stage sets in on the fifth to sixth day and on the tenth day flies begin to emerge. The development occurs equally well in beer-wort-agar enriched with vitamin $B_2$ whether the medium was or was not inoculated with acetic acid bacteria (Table II). The flies bred in tubes containing medium enriched by $B_2$ are fully fertile. Thus it may be affirmed that the development of Drosophila on beer-wort-agar inoculated with *Bacterium pasteurianum*

is impeded by vitamin $B_2$ deficiency. Acetic acid bacteria act as yeast in that respect only that similar to yeasts they stimulate the laying of eggs. In other words, the disturbance in the normal course of development is due to $B_2$ avitaminosis.

### Development under Conditions of an Avitaminous $B_2$ Régime

As mentioned above, the larvae raised on avitaminous $B_2$ food at a temperature of 25° C exist as such for a period from 18 to 22 days but the great majority of them die without reaching the pupal stage. The experimental results are not influenced by the density of the population: larvae die even if bred singly. The imaginal stage is reached by scarcely 1 per cent. of the flies (Table III).

It might be surmised that in experimental cultures the great majority of larvae die owing to the decrease of water contents in the medium—inevitable in the case of a prolonged development. However, the erroneousness of this hypothesis was demonstrated by specially undertaken experiments in which every 5 days the larvae under aseptic conditions were transferred to fresh tubes containing an avitaminous $B_2$ medium. Such larvae died likewise after a period of 18 to 20 days.

The progeny of individuals reared up to the imaginal stage on beer-wort-agar that was inoculated with *Bacterium pasteurianum* was studied. The idea was that in the case of such a progeny showing a better development than the preceding generation, when bred in the same unfavorable circumstances, that is, if the percentage of emerging flies were higher than is usually the case in the white strain,[2] this would mean that in our experiments selection had taken place. As a matter of fact, however, the development of an avitaminous $B_2$ medium of the progeny of flies bred under the same conditions had a

[2] The question in how far the avitaminous $B_2$ medium we have experimented with is fit for the development of other Drosophila strains is not discussed in the present paper. It suffices to remark that in this respect the white strain presents by far no exception.

course in no way more favorable than that of larvae in control cultures and it may be concluded that the few individuals which in our experiments attain the imaginal stage do not differ in their hereditary properties from other individuals of the same strain (Table IV).

Although the individuals that have attained the imaginal stage under conditions of an avitaminous $B_2$ régime do not differ in their hereditary properties from other individuals, still it is quite certain that they have suffered in a lesser degree: they lived while the others died (this in effect is a sort of tautology). We sought to save some of the individuals that suffered from unfavorable conditions to such an extent that they could not go over into the pupal stage. As mentioned above, some of the individuals live in their larval stage 18 to 22 days. The question is whether they might reach their final development if at that advanced date their nutritional régime were modified. By bringing a yeast medium into tubes containing 20-day old larvae bred under conditions of an avitaminous $B_2$ régime evidence was obtained that an addition of yeast saves the 20-day old larvae from death. But the development takes an unexpectedly slow course. The pupal stage is reached after 5 to 6 days and the flies begin to emerge on the ninth to tenth day, that is, 30 days after the eggs had been laid. The fact that the time elapsed from the moment when yeast was added to the medium up to the emerging of the flies coincides with the normal duration of development in *D. melanogaster* (10 days) renders it probable that in most larvae bred under conditions of an avitaminous $B_2$ régime, the process required for metamorphosis to occur is paralyzed from the very beginning of the larval period. This is the more probable, as not only the duration of development, but equally the duration of the larval stage coincide. It may also be noted that the 20-day old larvae are 3 to 4 mm long. Thus it may be concluded that the growth of the larva and its preparing for metamorphosis, leastways under the conditions applied in our experiments, are inde-

pendent of each other (it might perhaps be appropriate to repeat that both the experimental cultures and the control cultures were kept at 25° C and the temperature was the same for the cultures to which yeast was added after 20 days).

As mentioned above, the eggs are laid in equal numbers both in tubes containing beer-wort-agar inoculated with *Bacterium pasteurianum* as in the control tubes inoculated with yeast. At the time of addition of a yeast medium, that is, 20 days after the beginning of the experiment, in different tubes the number of larvae capable of continuing their development is most varied, the variability coefficient being much higher than in the controls. This means evidently that the death of the larvae in experimental tubes is in the various tubes not completely synchronous in its occurrence. In the average about one third of the larvae bred on avitaminous $B_2$ régime reach the imaginal stage (Table V). Compared with what was found for the cultures bred without subsequent addition of a yeast medium this percentage must be considered as being very high. Apparently the method described (addition of a yeast medium) actually makes it possible to use for genetical purposes part of the individuals that have been highly influenced by unfavorable developmental conditions.

Under conditions of an avitaminous $B_2$ régime the great majority of larvae do not go over into the pupal stage. But it suffices for a freshly hatched larva to live for 24 hours (or even 16 to 18 hours) on a sterile yeast medium—to say nothing of longer periods—for such a larva to prove able of changing into a pupal on being brought onto beer-wort-agar. Larvae bred for a period not above 24 hours on a yeast medium continue their development on an avitaminous $B_2$ medium and go over to the pupal stage on the fifth to sixth day counting from the time when the eggs were laid. The flies emerge on the tenth day. The emerging of flies is of 100 or about 100 per cent. (Table VI). Control larvae that from the very beginning were

bred on an avitaminous $B_2$ medium, as is usually the case, die sooner or later without changing into pupae.

### CERTAIN CHARACTERISTICS OF THE IMAGO

For further investigations four groups of individuals could be employed: (1) control individuals grown on a yeast medium, (2) individuals which were given a yeast medium on the twentieth day of their larval period, (3) individuals that after hatching were placed in a yeast medium for a period of 24 hours at the utmost and continued their development in an avitaminous $B_2$ medium, finally, (4) the few individuals which reached the imago stage under conditions of an avitaminous $B_2$ régime.

The individuals belonging to the second group do not markedly differ from their controls whereas the individual of the fourth group, and especially those of the third group differ sharply from their controls: they are very small, very feeble and they frequently lack the strength to fully throw off their pupal sheath. We studied the duration of life and fertility of the individuals in all four groups. Our purpose was to establish by comparing the data obtained how far the changes produced in the larvae by the various conditions of development are to be considered as important. Males alone were studied as it is precisely the males that were subsequently used for analysis of the mutational process.

In studying the longevity of the control males we brought the males alone without females 4 to 6 hours after they had emerged into sterile tubes containing a yeast medium; five males for each tube. The cultures were kept from 12° to 14° C. Every 10 days the flies were transferred into fresh tubes. The males of the three other groups were kept in the same conditions. As in Table VII, the individuals of the second group, to which on the twentieth day of larval life yeast medium was given, do not differ from the controls. In the experiment about 90 per cent. of them were found to live as long as 50 days (observations were then discontinued). Of the fourth

group, flies bred in an avitaminous $B_2$ medium less than 20 per cent. grew to be 50 days old. Finally the flies in the third group, in which the larvae after hatching were bred for 24 hours at the utmost in a yeast medium, presented the greatest differences from the controls—not one of them lived as long as 50 days.

When fertility was studied similar results were obtained (Table VIII). The second group flies were found not to differ from the controls, whereas those of the third group were more strikingly altered than the flies in the fourth group. The third and fourth group flies are in part infertile in the first days after emerging (these make out about one third of the numbers in the fourth group and about one half of the third), later on however they become fertile.

Basing on these data and on the facts discussed in the previous pages of the present paper we are led to the following conclusions:

It suffices for vitamin $B_2$ to be present in minimal quantities for the pupal stage to set in and the emerging of the flies to occur. This is why in the third group, in which the larvae just hatched from the egg were given a yeast medium for a period not surpassing 24 hours (whereupon they were subjected to an avitaminous $B_2$ régime), 100 per cent. emerge. But vitamin $B_2$ influences not metamorphosis only, the organism as a whole likewise is strongly affected; thus individuals which in their larval stages were given the vitamin in insufficient quantities or were fully deprived of it (the third and fourth groups) differ sharply from the controls not only in respect of external characters such as size but also as regards longevity and fertility.

The second group forms that were given vitamin $B_2$ in unrestricted quantities on the twentieth day of their larval life are indistinguishable from the controls both in their outer characters in respect to their longevity and fertility, although before addition of the yeast medium the larvae were bred for a period of 20 days on avita-

minous $B_2$ food. The individuals of the third group differ more sharply from the controls than those of the fourth. The reason for this lies in that the fourth group is composed of but a small number of individuals that have reached the imago stage (the death percentage is 99 per cent.) whereas the third groups show a 100 per cent. emergence of flies so that in this group individuals that have suffered least must be in the minority.

### SEX LINKED MUTATIONS OF THE SECOND GROUP

The study of the "spontaneous" mutational process was started in the autumn of 1936 by a comparative examination of the frequency of mutations in the X chromosomes in control males bred in a yeast medium, and in second group males (these received vitamin $B_2$ food after a larval period of 20 days in which the larvae were kept under condition of an avitaminous $B_2$ régime). In the second group, as mentioned above, the development at 25° C lasts 30 days. In respect of longevity and fertility the imaginal forms do not differ from the control group. Nevertheless, such flies in their larval periods were highly influenced by unfavorable developmental conditions (more so than the individuals of group four which reached the imaginal stage without having a yeast added to their nutritive medium). Therefore, these forms represent an adequate material for studying the mutational process as it takes place under condition of an avitaminous $B_2$ régime.

The frequency of mutations occurring in the sex chromosomes was determined by way of the C1B method. Experimental and control males were crossed the day of their emergence with $\frac{C1B}{scvfcar}$ females, and two days later the parent flies were removed from the tubes. Only such mutations were considered which effected a 100 per cent. death of the males in the second generation. The mutations that arose were all verified in the third generation.

The data obtained are given in Table IX. The percentage of mutations seems to be about the same both in the experiment and in the control; it is somewhat lower in the experimental material, but the difference is not in the least valid. Diff. $= 0.06 \pm 0.11$. This result, however, has quite another meaning considering that the second group flies take 30 days to develop—a period three times as long as that observed in the controls (10 days). If the "spontaneous" mutations arise autonomously, *i.e.*, independently of the developmental conditions, as assumed by many geneticists, if independently of the life of the individuals, it were possible to speak of periods that separate the time which elapses between two mutations of a gene, if the number of the mutations arises spontaneously were simply a function of time (such is the point of view that figures in most general articles on the subject—for instance Timofeiev-Ressovsky, 1937), then the percentage of mutations in the second group males would be three times as high as in the control. However, it might be said in favor of the generally accepted opinion, denying the existence of a connection between the mutational process and the development of the individual, that the diminished percentage of mutations per time unit observed in the experimental males may be dependent not on a decrease in the number of mutations that arises per time unit but on a process of elimination of the mutated cells. In that case the decrease in the number of mutations occurring per time unit in the experiment as compared with the control should be considered as due to germinal selection. At present the existence of germinal selection is accepted by most investigators (still it ought to be mentioned that up to now the authors who studied this process have not worked with "spontaneous" mutations). In our case it might suffice to admit that in individuals developing under the unfavorable conditions of an avitaminous $B_2$ régime germinal selection is more intense, *i.e.*, that the germ cells in which sex-linked lethals make their appearance die much more frequently or multiply more slowly than the analogous cells in the control males.

AUTOSOMAL MUTATIONS IN THE SECOND GROUP MALES

With the view of rendering more complete our data on the "spontaneous" mutational process and at the same time of verifying the acceptability of the germinal selection hypothesis as an explanation of the results already obtained, the "spontaneous" mutational process as occurring in chromosome II was studied. Here also the analysis was carried out on control males and on second group males which had been given a yeast medium on the twentieth day of their larval life. The $\frac{\text{CySp}}{\text{L}^2}$ method was employed as it helps to bring into evidence in the third generation such lethal mutations as took place in chromosome II in the experimental as well as in the control males. The only mutations considered were those which in the third generation produced death of all forms homozygous with regard to the mutation investigated. All mutations were verified in the fourth generation.[3]

The data obtained (Table X) showed that the percentage of lethal mutation found in the chromosomes II of experimental males differs but slightly from that found in the controls, it is even somewhat lower. Statistically the difference is not quite real. Diff. $= 0.41 \pm 0.21$. At the same time, supposing that the number of mutations arisen corresponded with the period of time elapsed, the mutations would have been three times as numerous in chromosome II of the second group males of 30 days' development as compared with the controls which took but 10 days to develop. Counts were made in order to obtain evidence as to how far the difference was statistically real that was noted between the number of mutations actually found in the experimental males and the

[3] Verification of the cultures which were found to contain non*Cy* flies in single numbers is made possible owing to the presence of the *Sp* gene in the strain used for analysis. It was found that the appearance of individuals of this sort may be due to crossingover of the *CySp* chromosome with the chromosome bearing the newly arisen lethal; the percentage of crossovers never surpasses 1 per cent. Such strains were naturally considered as bearing a lethal mutation.

number of mutations to be expected if the rate of the mutational process were independent of the peculiarities of the individual's development, given that, the number of mutations arising per time unit in experimental males is the same as in the control males. Now, the number of mutations found in the control, $M^c = 42 \pm 6.45$. By subtracting from it three times its average error the minimal number for the mutations possible in the control is obtained, $M^{c\ min.}$. $M^{c\ min.} = 42 - 6.45 \times 3 = 22.65$. What then would be the smallest number of mutations expected in the experiment ($M^{exp.\ probable\ min.}$) if the rate of the mutational process in experimental males were the same as in the control? It would evidently coincide with the minimal number of mutations in the control ($M^{c\ min.}$) multiplied by 3 (the experimental males take three times longer to develop than the controls) and again multiplied by 1.04 (in the experimental material a somewhat larger number of chromosomes was studied than in the control). Hence $M^{exp.\ probable\ min.} = 22.65 \times 3 \times 1.04 = 70.07 \pm 8.30$. The number of mutations actually found in the chromosomes of the experimental males ($M^e$)$= 27$. Therefore, the difference between the number of mutations actually found in the experimental males and the number to be exepcted, if the rate of the mutational process were independnt of the peculiarities of the development of the individual, is quite real statistically. Diff. $= 43.07 \pm 8.30$.

We arrive at the same conclusion by applying the $X^{second}$ method. In the case of an alternative variation, if $X^2 = 85.3$ the probability that the difference between the expected and the actually found number of mutations in the experiment represents a chance difference is 0.0I (according to Fisher's tables).

It must be noted that this conclusion retains its force even in the case if, in computing the expected number of mutations in the experiment, we start from the minimal probable number of mutations in the control which is 22.65 . $X^2$ will then be 26.9 and, therefore, the above conclusions remain valid.

The study of the mutational process in chromosomes II of the second group males helped to confirm, and gave statistical certainty to, the conclusion already indicated in the analysis of the mutational process in chromosome X: namely, that the rate of the mutational process, *i.e.*, the number of mutations arising per time unit, is dependent on the conditions in which the individuals develop. Moreover, this conclusion can not now be subjected to doubt by a reference to germinal selection. The recessive lethal mutations that arise in chromosome II, contrary to the sex linked mutations, remain heterozygous up to the reduction division. Germinal selection is, therefore, unable to occasion a strong decrease in the number of mutations found. At the same time the percentage of mutations found in the experiment as related to that in the controls differs but slightly for chromosomes X and II, the decrease in the number of mutations in the experiment (in so far as it is possible to speak of such a decrease) as compared with the controls is even somewhat more pronounced in chromosome II (Tables IX and X).

TABLE IX

LETHAL MUTATIONS IN THE X CHROMOSOMES OF CONTROL AND EXPERIMENTAL SECOND GROUP MALES

| Control | | | Experiment | | |
|---|---|---|---|---|---|
| Number of chromosomes studied | Number of lethal mutations | The same in per cent. | Number of chromosomes studied | Number of lethal mutations | The same in per cent. |
| 352 | 1 | | 730 | 1 | |
| 676 | 1 | | 741 | 1 | |
| 489 | 1 | | 944 | 2 | |
| 880 | 2 | | 817 | 1 | |
| 2,397 | 5 | 0.21 ± 0.08 | 3,232 | 5 | 0.15 ± 0.07 |

Diff. = 0.06 ± 0.11.

TABLE X

LETHAL MUTATIONS IN THE II CHROMOSOMES OF CONTROL AND EXPERIMENTAL SECOND GROUP MALES

| Control | | | Experiment | | |
|---|---|---|---|---|---|
| Number of chromosomes studied | Number of lethal mutations | The same in per cent. | Number of chromosomes studied | Number of lethal mutations | The same in per cent. |
| 832 | 9 | | 648 | 4 | |
| 695 | 7 | | 837 | 5 | |
| 938 | 10 | | 869 | 6 | |
| 720 | 6 | | 903 | 6 | |
| 239 | 4 | | 338 | 3 | |
| 511 | 6 | | 494 | 3 | |
| 3,935 | 42 | 1.07 ± 0.16 | 4,089 | 27 | 0.66 ± 0.13 |

Diff. = 0.41 ± 0.21.

TABLE XI

LETHAL MUTATIONS IN THE II CHROMOSOMES OF CONTROL AND EXPERIMENTAL THIRD GROUP MALES

| Control | | | Experiment | | |
|---|---|---|---|---|---|
| Number of chromosomes studied | Number of lethal mutations | The same in per cent. | Number of chromosomes studied | Number of lethal mutations | The same in per cent. |
| 941 | 7 | 0.74 | 688 | 5 | 0.73 |

Diff. = 0.01 ± 0.43.

The decrease in the rate of the mutational process in the second group males as compared with the control, *i.e.*, the diminished number of mutations arising per time unit, reflects in itself the influence of the conditions that determine the peculiarities of the individual's development, but have no direct effect on the germ cells.

Various hypotheses may be proffered for the explanation of this influence. Thus, it may be supposed that an avitaminous $B_2$ régime lowers the intensivity of metabolism and that the direct effect of it is a decrease in the rate of the mutational process.

However, previous to a discussion of the comparative value of such hypotheses attention must be drawn to a fact of obviously considerable import. How is it that by reason of a decrease in the rate of the mutational process in the second group males the percentage of mutations in relation to the number of chromosomes studied is about the same in the experiment and in the control? Might not this fact indicate that in ontogenesis causes are operative that produce accumulation of a certain number of mutations in the individual's germ cells at the time of the emerging of the flies?

### AUTOSOMAL MUTATIONS IN THE THIRD GROUP MALES

The solution of the question as to what occasions the decrease in the rate of the mutational process in the second group males as compared with that in the control males would have presented great difficulties had we not at our disposal the third group individuals bred directly after hatching on a yeast medium for a period of 24 hours at the utmost, the subsequent development taking place

in an avitaminous $B_2$ medium. In the third group, owing to the circumstance that in it a 100 per cent. emerging of flies takes place, the unfavorable effect of the avitaminous $B_2$ medium is most pronounced. In it, however, development is achieved in 10 days, just as in the control group. Therefore, by studying the third group males an answer may be obtained to the question whether the decrease in the rate of the mutational process noted in the preceding experiments is due to the prolonged development of the second group forms or whether it is due to other causes (such as decrease in metabolic intensivity).

The analysis was carried out solely on third group males that were infertile during the first four days after emerging, that is, on such individuals which were most highly influenced by the unfavorable action of the avitaminous $B_2$ régime. Similarly the control males were employed on the fifth day after emerging. In this series, as in the preceding experimental series, the $\frac{CySp}{L^2}$ method was applied for the study of the "spontaneous" mutational process in chromosome II. The technique of recognizing new mutations was the same as in the previous experiments.

The results obtained in this experiment are given in Table XI. It shows that the percentage of mutations noted in chromosome I in the third group males equals the percentage of mutations found in the control.

Thus we may consider as proved that the decrease in rapidity in the mutational process, brought into evidence in the preceding experiments, in which males of the second group were studied, is due to the fact that in the group under discussion the development is of longer duration. The decisive rôle may quite possibly belong here to a slowing down of the process of spermatogenesis, or, more exactly, in connection with the latter, to a slowing down of the rate of reproduction ("multiplication") of the genes.

SUMMARY

The results obtained in the present paper enable us to conclude that the number of mutations formed in an individual's germ-cells up to the close of metamorphosis is characteristic of the corresponding period of life of the individual. In our opinion this fact proves the erroneousness of the current view that the spontaneous mutation process is autonomous, *i.e.*, independent of the development of the individual.

# REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

## RECENT BOOKS ON GENETICS

(1) **An Introduction to Modern Genetics,** by C. H. Waddington. New York: The Macmillan Co., 1939: 1–441, 5 plates and 160 figs. $4.00.

(2) **An Introduction to Genetics,** by A. H. Sturtevant and G. W. Beadle. Philadelphia and London: W. B. Saunders Co., 1939: 1–391, 126 figs. $3.25.

(3) **Mammalian Genetics,** by W. E. Castle. Cambridge, Mass.: Harvard University Press, 1940: i–viii, 1–169. $2.00.

(4) **Manual for a Laboratory Course in Genetics** (to accompany "Mammalian Genetics"), by W. E. Castle. Cambridge, Mass.: Harvard University Press, 1940: 1–21, 7 tables. $0.40.

(5) **Genetics,** by H. E. Walter. Fourth Edition. New York: The Macmillan Co., 1938: i–ix, 1–412. $3.00.

(6) **Elements of Genetics,** by E. C. Colin. Philadelphia: The Blakiston Co., 1941: i–xii, 1–386. $3.00.

(7) **Suggestions for Teaching Selected Material from the Field of Genetics,** by A. D. Laton and E. W. Bailey. Monograph No. 1, Bureau of Publications, Teachers College, Columbia University, New York, 1939: i–viii, 1–66. $0.50.

(8) **An Experiment in the Teaching of Genetics,** by A. De M. Bond. Contributions to Education, No. 797, Bureau of Publications, Teachers College, Columbia University, New York, 1940: i–viii, 1–99. $1.85.

(9) **Studies on the Genetics of Drosophila,** directed by J. T. Patterson. The University of Texas Publication No. 4032. Austin, Texas, 1940: 1–256.

(10) **Bibliography on the Genetics of Drosophila,** by H. J. Muller. Edinburgh: Oliver and Boyd, 1939: 1–132. 5 shillings.

(11) **Organisers and Genes,** by C. H. Waddington. Cambridge: at the University Press, and New York: The Macmillan Co., 1940: 1–160. $3.00.

A SURVEY of eleven recent books, each containing in its title the word "genetics" or "genes," shows quite definitely that genetics is more than the "study of heredity." Descriptive analysis of Mendelian inheritance has largely given way to newer studies in which the identification of a gene marks only the starting point of the investigation. Recent books on genetics therefore show a tendency to reduce the treatment of formal Mendelism in favor of the newer specialties of cytogenetics, physiological genetics and population genetics. Likewise, in the more elementary texts one finds less apology for the gene theory and more space devoted to practical applications.

Outstanding for its comprehensive treatment of these special topics, and therefore especially useful as a summary for biologists in other fields, is C. H. Waddington's "An Introduction to Modern Genetics" (1). The volume is arranged in five parts: I. Formal Genetics; II. Genetics and Development; III. Genetics and Evolution; IV. Genetics and Human Affairs; and V. The Nature of the Gene. The basic facts concerning genes and chromosomes are concisely but adequately described in the first 133 pages. In the remaining sections the author has supplied excellent summaries on such topics as genes and development, the effects and interactions of genes, the genetic nature of taxonomic differences, evolutionary mechanisms, the mathematical theory of evolution, the nature of the gene and special aspects of human genetics. Specialists will inevitably criticize the treatment within these separate fields, but they will almost certainly admit that each branch has received its due consideration and is placed in proper perspective to the subject as a whole. It should be emphasized, however, that Dr. Waddington's text was written for the biologist and not for the practical student of agriculture, sociology or medicine. The 160 text figures appear to have been specially prepared and each has been provided with a very full explanatory heading, so that a fairly good review of genetics might be obtained from the figures alone. Excellent plates of chro-

mosomes and a good bibliography and index add much to the value of this book, which might well be taken as a model for other "modern" accounts.

If one still chooses to think of the geneticist as the student of heredity, then it is probably the cytogeneticist who can lay best claim to that title, since he deals with the ultimate inheritance of organisms. Written from this point of view, "An Introduction to Genetics" (2) by the *Drosophila* geneticists, A. H. Sturtevant and G. W. Beadle, stresses the experimental approach and the material basis of inheritance. Commencing with a discussion of sex chromosomes and sex linkage, the chromosome remains the center of interest throughout a large share of the book, and considerable attention is paid to polyploids and chromosomal aberrations. Although the scope is thus somewhat advanced, the material is presented in a clear and orderly fashion, and numerous well-devised problems are included in each chapter. The text was obviously designed for a beginning but technical course rather than a more popular general course. Most of the examples are chosen from *Drosophila* and little effort has been made to relate the study to plant, animal or human variations with which the ordinary student is apt to be familiar.

Designed to meet this latter objection is the very attractive volume by Professor Castle on "Mammalian Genetics" (3), for which an accompanying laboratory manual (4) is also available. As more and more becomes known about genetic variation throughout the animal and plant kingdoms, it is to be expected that whole books will be devoted to particular groups. Whether or not such specialization is advisable in a text-book seems questionable. The biologist, desirous of demonstrating the generality of his science, has no better opportunities than those afforded in genetics. Although Professor Castle's book might serve as a text if supplemented by other material, its usefulness as a "survey of present knowledge of the genetics of mammals" is more apparent. A comparative

survey of genetic variation, particularly the pathological, in mammals should prove very helpful to the animal breeder and medical geneticist. A beginning to such a book is found in "Mammalian Genetics," which lists and compares the better-known (mostly non-pathological) gene mutations of the laboratory rodents and a few other species.

Two text-books intended for the more elementary course are furnished in H. E. Walter's "Genetics" (5) and E. C. Colin's "Elements of Genetics" (6). These volumes are quite similar in style, scope and objectives. The first edition of Professor Walter's "Genetics," published in 1913 was among the earliest accounts of the new science to appear in this country. The new fourth edition has undergone a complete rewriting and exhibits a good deal of originality in examples, illustrations and methods of presentation. Teachers of genetics and allied subjects should find some useful teaching devices in this book. Like Professor Walter, Dr. Colin uses the historical approach and comments frequently on personalities. "Elements of Genetics" includes a large amount of material on man. In two long chapters the author has prepared a rather extensive list of hereditary conditions in man, similar to the more complete treatment in a recent book by Professor Snyder (to be reviewed in January).

"Suggestions for Teaching Selected Material from the Field of Genetics" (7) gives recommendations on approaches, objectives, subject matter and methods of evaluation of teaching results in an elementary course. "An Experiment in the Teaching of Genetics" (8) by A. De M. Bond contrasts the effectiveness of two courses, one stressing the implications of genetics for human welfare, the other presenting the more conventional, factual material. The results indicated that changes in certain attitudes, while occurring in the conventional course, were increased in the experimental course, and to some extent without reduction in the gain of factual knowledge. These two studies from the Columbia University Bureau

of Educational Research reflect the growing popularity of genetics as an applied science in the field of general education.

Two publications of very great value to the large society of *Drosophila* workers have recently been made available. The first is a series of "Studies on the Genetics of Drosophila" (9) by Dr. J. T. Patterson and several of his former and present co-workers at the University of Texas. The general topics discussed in this series include chiasmata formation, non-disjunction, multiple sex genes, artificially produced aneuploidy, position effect, hybridization and evolution. Together they constitute a more or less connected story and will certainly prove of interest to many workers outside the field of *Drosophila*. Professor Muller's very complete "Bibliography on the Genetics of Drosophila" (10) has been eagerly received since its publication in 1939. Since a similar list was published in 1924 the literature on this fly, mostly cytological and genetical, has increased seven-fold, and the present list now contains 2,965 titles.

Although *Drosophila* remains a favorite subject for cytological and genetical research, insects in general have not proven very suitable for experimental studies in embryology. One might wish that more were known genetically about the echinoderms and Amphibia, which have served so well in the hands of experimental embryologists. A comparison of basic principles in these fields might therefore be made less difficult. Dr. C. H. Waddington feels that an attempt should nevertheless be made to integrate the experimental work in embryology and genetics; to compare, as it were, the effects of mixing different somatoplasms with the effects of mixing different germplasms. Such an attempt is made in "Organisers and Genes" (11), which represents Dr. Waddington's own contribution to the current series of Cambridge Biological Studies, of which he is editor.

The essay begins with a description of organizers, the development-controlling regions of embryos which were

first revealed in the classical work of Spemann dating from 1913. A significant advance was made when it became apparent that the process of developmental determination depends both on the reactivity (competence) of the host tissue and the presence of an activating substance (evocator) produced by the organizer. After reviewing some very interesting work on the chemistry and physiology of organizers, the author then undertakes an analysis and classification of genic action. A parallelism is demonstrated in the various effects of organizers and genes and the conclusion is reached that genetic factors control development by affecting the competence of the cytoplasm and not by controlling the evocators. Competence is described as a state of instability, a readiness of the tissue to react in two or more different ways. The genotype thus determines the most probable alternative to be followed at each step in a long "branching track" of development.

"Organisers and Genes" makes rather difficult reading, and a more concise statement of the author's views will be found in the sixth chapter of his "Introduction to Modern Genetics." Despite its philosophical character, however, the book is not likely to vex the more practical-minded biologist. The author takes care to point out that many of his classifications are arbitrary and are advanced wholly for the purpose of pointing the way to further experimentation. No great claims are made for his own points of view, but considerable optimism is held for the future. Denying the thesis that biological differentiation is incompatible with physical-chemical analysis, Dr. Waddington suggests that logicians and topologists may have to come to the rescue by devising a more precise language for dealing with problems of biological patterns and organization.

CHAS. W. COTTERMAN

**Medical Genetics.** By LAURENCE H. SNYDER. Durham, N. C.: Duke University Press, 1941: i–viii, 1–130, 24 figures, 12 tables. $1.50.

A NUMBER of physicians have stated that they would like to know something about heredity, but at the first mention of a fruit-fly or the first flight into higher mathematics they toss the book away. These two stumbling blocks have now been removed.

In the spring of 1941 Dr. Snyder delivered, under the auspices of the Carnegie Corporation, a series of lectures in medical genetics at the three medical schools in North Carolina. These talks were so well received that they have been put in book form at the request of faculty members of these schools.

In this series of lectures Dr. Snyder has succeeded in bringing together, in a well-organized and highly usable form, the essentials of a vast amount of investigation in the field of inherited disease. As this book is intended primarily for physicians and medical students with no special training in genetics, the traditional approach to the basic principles has been abandoned. The major types of genetic mechanism are briefly explained in connection with specific disease traits as these traits appear in an orderly classification.

In his opening chapter Dr. Snyder discusses the practical applications of genetics to medical practice, emphasizing the interaction of heredity and environment in the causation of disease, and explodes a number of widely held misconceptions regarding hereditary diseases in general.

A concise discussion of the medicolegal applications of blood groups and blood types is followed by a consideration, system by system, of the diseases in which heredity is known to play a major rôle. These conditions are grouped into: mental disorders; eye and ear abnormalities; abnormalities of the skin; skeletal and muscular abnormalities; diatheses and susceptibilities (including diabetes mellitus, hypertension, allergies and others); diseases of the blood (grouped as hemorrhagic diatheses, anemias and storage diseases); and cancer. The essential clinical features of each disease are described briefly,

and the mode of inheritance discussed. An outstanding feature of the presentation is the use of tables classifying the diseases of each system and showing the hereditary entities in relation to the entire field. The concepts of penetrance and expressivity are clearly presented. The difficult subject of cancer is well treated in the limited space available and the evidence for a tendency to tumor production is summarized.

In the final chapter, in which the effects of genes borne on the sex chromosomes are summarized, Dr. Snyder considers the interesting possibilities in medical research and therapy which further investigation in human genetics is likely to present. Studies in autosomal linkage may provide clinicians and pathologists with the opportunity of observing the development of disease before actual symptoms appear. It may also become possible to institute active therapy in the preclinical stages of certain conditions.

This book should become most useful in the hands of the practicing physician, but will also be of considerable value to any one interested in the practical applications of heredity in man. An adequate index enhances its value as a reference work.

C. Nash Herndon

**Human Biology.** By George Alfred Baitsell. McGraw-Hill Book Co., 1940: i–xv, 1–621, pls. 1–18, figs. 1–260. $3.75.

A late-lamented colleague with a horticultural hobby was once inspecting what a lady regarded with pride as her very lovely rock garden. Turning aside to me, he remarked, "This may be a garden with rocks in it, but it is *not* a rock garden." So I might say that "This book may be a biology with man in it, but it is *not* a human biology." It does not in any way fill the need for general treatises and general training in the biology of man, but it *is* a biology—and a very good one. It was written on a premise, which seems to have a firm basis in educational psychology, that there is an advantage in focusing

the elementary college course in zoology or general biology on a subject of initial, general and keen interest, man himself. The extensive text is interestingly written and abundantly clarified with well-selected and original illustrations, and is logically organized and broad in its coverage. The Appendix is a brief but helpful encyclopedia of biology, largely in the form of quotations.

**The Orientation of Animals. Kineses, Taxes, and Compass Reactions.** By GOTTFRIED S. FRAENKEL and DONALD L. GUNN. Oxford and New York: Oxford University Press, 1940: i–viii, 1–352, figs. 1–135. $6.00.

THOUGH dealing essentially with the mechanism of orientation, with particular emphasis on the classification and nomenclature of responses, this needed volume on "The Orientation of Animals" has an evolutionary background. The responses of animals in posture and movements are repeatedly stated to have adaptive significance. Through Part I orientations of increasing complexity are treated in turn: "ortho-kineses," involving different degrees of hit-and-miss movement, varying with the intensity of the stimulus; "klino-kineses" (better "clino-kineses"?)—the trial-and-error responses in which the frequency of turning is the variable; "klino-taxes," including similarly irregular but directed movements; "tropo-taxes," in which the definitely directed responses, operating through two receptors, are balanced; "telotaxes"—similar reactions in which a single receptor dominates the action at any one time; "light compass reactions," in which any response is at a certain angle to the stimulus; "dorsal (and ventral) light reactions" and "ventral earth reactions," causing the animal to orient itself at right angles to light and gravity. Experimental results with these and other types of orientations are treated in some detail in Part II, which seems more designed for the investigator than the student. The interpretations are on a mechanistic basis, though Loeb's reduction-to-the-simplest explanations of animal movements are not agreed to. The terminology is adopted,

and in part propounded, in an effort (not wholly successful) to eliminate the anthropomorphic attitude. Some workers on animal behavior will no doubt feel that too much attention is given to the classification and nomenclature of animal reactions. The authors freely admit that difficulties are involved in the identification of the many types of response according to their own scheme, which is a modification of Kühn's classification of 1919.

## News Items

The Association for the Study of Systematics in Relation to General Biology is continuing to function despite the conditions which war has brought to England. During the year the association issued its second report, for 1938–40, in which there are listed 14 investigational projects initiated or assisted by the Research Committee, and 4 symposia arranged or suggested by the association, including "A Discussion on Phylogeny and Taxonomy" published in the Proceedings of the Linnean Society (152, 1940: 234–55). Subsequently, on July 10, the association held a joint meeting with the Linnean Society to discuss "Differences in the Systematics of Plants and Animals and their Dependence on Differences in Structure, Function and Behaviour in the Two Groups." Following this symposium the annual general meeting was held. The association sponsored the publication of "The New Systematics," reviewed in the March–April number of The American Naturalist.

Genetics and speciation will be highlighted at the Christmas meetings in Dallas. The American Society of Zoologists is arranging or participating in four Symposia: (1) Isolating Mechanisms; (2) Temperature and Evolution; (3) The Genetic Control of Embryonic Development; (4) Human Genetics. The annual address of the vice-president of Section F of the A. A. A. S. will be delivered by Professor J. T. Patterson, who will speak on "Drosophila and Speciation."

THE GENETICS SOCIETY OF AMERICA (Th. Dobzhansky, president) will meet with the other biological societies affiliated with the A. A. A. S. in Dallas, Texas, from December 29 to 31. The tentative program of the society includes two sessions for the presentation of scientific papers by the informal type of demonstration that has characterized the meetings of the society during recent years. One session will be devoted to the reading of short papers (5 to 10 minutes), and another to the presentation of a small number of papers selected by the program committee for more extensive consideration. The luncheon and business meeting is scheduled for December 30. Several other groups are joining with the geneticists in sponsoring a symposium dealing with isolating mechanisms. The list of speakers includes G. Ledyard Stebbins, Albert P. Blair, Alfred C. Kinsey and J. T. Patterson. Professor Patterson has been asked to serve as chairman of the meeting. The tenth edition of the Records of the Society, containing the membership list, and the complete program together with preprints of abstracts of papers to be presented at the Dallas meetings will be sent to members of the society about December 15. Non-members can purchase copies of the Records from the secretary, B. P. Kaufmann, Carnegie Institution of Washington, Cold Spring Harbor, New York.

The annual meeting of the American Society of Naturalists, also at Dallas, will be highlighted by the Presidential Address by Dr. W. W. Cort, of Johns Hopkins University, on "Human Factors in Parasite Ecology." This will be given at the Wednesday evening Naturalists' Dinner, December 31, 6:30 P.M. Jointly with other organizations, the Society is sponsoring an important symposium on Human Genetics, scheduled for 2:00 P.M., December 31. The program is as follows:

1. The Human Gene. Laurence H. Snyder, Ohio State University.
2. The Genetics of Human Populations. Herluf H. Strandskov, University of Chicago.

3. The Biometrical Approach in Human Genetics. Charles W. Cotterman, University of Michigan.

4. The Future Possibilities of Human Genetics. Lionel S. Penrose, Ontario Hospital.

For the June meeting in Salt Lake City of the Pacific Division of the A. A. A. S., there is being arranged a symposium on the relation between postglacial climatic changes and the distribution and speciation of organisms in the now greatly desiccated Great Basin.

In the passing of Victor Jollos the science of evolution has lost one of its leading modern contributors. This scientist's eventful career, from his birth at Odessa in 1887 through wanderings and teachings in many lands until his death at Madison in July of this year, is briefly told in the September 19 issue of Science. He was trained by Robert Koch, and first attained prominence as a protozoologist. Perhaps the greatest of his contributions was to the conception of *Dauermodifikationen*. His work on the directive influence of external factors on mutation, however, has received the widest notice and the strongest criticism. It is of interest to learn that Jollos himself was unable to reproduce his original results.

# SHORTER ARTICLES AND DISCUSSION

## LINKAGE, INTERFERENCE AND SEMILETHALS IN THE WHITE GROUP OF HABROBRACON

### I Introduction

The study of linkage in the parasitic wasp *Habrobracon juglandis* (Ashm.) is aided by the fact that unmated females, which are diploid and arise from fertilized eggs, normally produce azygotic (haploid) males in gametic ratios. Thus, counts of $F_2$ males bred from heterozygous $F_1$ virgin females are sufficient to show the presence or extent of linkage. The azygotic ratios obtained, however, are subject to various modifications and limitations which have been enumerated by Whiting and Benkert (1934). Of these, the most important for the present work is the influence of lethal or semilethal factors linked to the visibles studied. It is not surprising that the actual ratios often show deviations which are due to lethals, as in such a haploid organism as the male Habrobracon any factor or condition producing a more or less lethal effect would have a maximum opportunity for expression due to the absence of any dominant allele to "cover" it. It is the purpose of this paper to present and to examine critically the data obtained from experiments on three linked visibles and to indicate the influence of linked lethals or semilethals on the ratios.

The writer is greatly indebted to Dr. P. W. Whiting for suggesting the problem and for his invaluable assistance in the interpretation of the data. Technical help has been received through a grant to Dr. Whiting from the Carnegie Corporation of New York.

### II Materials and Methods

Crosses were made in which the recessive factors broken, *bk* (wings), white, *wh* (eyes) or its allele carrot, $wh^c$, and stumpy, *st* (legs) were introduced in the four possible ways with respect to each other. In addition to crosses involving these three factors, the data from a linkage test of *bk* and $wh^c$ with shiny, $gz^s$ (eyes) are presented. The factor *bk* is characterized by irregularities in width of the vein along the costal margin of the wing distal to the stigma. It is usually asymmetrical in expression and often accompanied by a pronounced notch at

TABLE I

PROGENIES OF THREE-POINT CROSSES IN THE WHITE GROUPS*

| Experiment | I | II | III–a | III–b | IV–a | IV–b |
|---|---|---|---|---|---|---|
| $F_1$ ♀♀ / $F_2$ ♂♂ | *(1) + (2)* | *bk (1) (2)* | *bk (1) whe (2)* | | *(1) wh (2)* | |
| | *bk* *whe* *st* | *whe* *st* | *st* | | *bk* | *st* |
| + | 872 *(–)* | 231 *(1)* | 115 *(2)* | 18 *(2)* | 7 *(1, 2)* | 7 *(1, 2)* |
| *wh* *bk whe st* | 817 *(–)* | 190† *(1)* | 60 *(2)* | 14 *(2)* | 1 *(1, 2)* | 3 *(1, 2)* |
| *wh* *whe st* | 228 *(1)* | 442 *(–)* | 27 *(1, 2)* | 2 *(1, 2)* | 55 *(2)* | 64 *(2)* |
| *bk* | 228 *(1)* | 434 *(–)* | 2 *(1, 2)* | 3 *(1, 2)* | 53 *(2)* | 43 *(2)* |
| *st* | 94 *(2)* | 6 *(1, 2)* | 882 *(–)* | 163 *(–)* | 161 *(1)* | 130 *(1)* |
| *wh* *bk whe* | 96 *(2)* | 3 *(1, 2)* | 607 *(–)* | 170 *(–)* | 106 *(1)* | 99 *(1)* |
| *wh* *whe* | 12 *(1, 2)* | 73 *(2)* | 521 *(1)* | 67 *(1)* | 562 *(–)* | 438 *(–)* |
| *bk st* | 3 *(1, 2)* | 78 *(2)* | 133 *(1)* | 51 *(1)* | 456 *(–)* | 424 *(–)* |
| Totals | 2350 | 1457 | 2347 | 488 | 1401 | 1208 |
| % crossovers, region 1 | 20.0 | 29.5 | 29.0 | 25.2 | 19.6 | 19.8 |
| % crossovers, region 2 | 8.7 | 10.9 | 8.7 | 7.6 | 8.3 | 9.7 |
| % of coincidence | 36.5 | 19.0 | 49.0 | 53.5 | 35.1 | 43.2 |

* For explanation, see text.
† Includes 50 *whe st* individuals indeterminable for *bk*.

the tip of the wing. The recessive shiny, *gz*$^s$, is characterized by an extreme glossy appearance of the eye unaccompanied by the deleterious effects of glazed, *gz*.

The factors *bk*, *wh*, *wh*$^e$ and *st*, with which most of the work was done, are very favorable for the study of linkage and lethals as there are (1) no masking effects, (2) no overlappings of one class with another except for a possible insignificant overlapping of *bk*, (3) no viability differences, save for a slightly reduced viability of the triple recessive and the minor difficulties experienced by the *st* individuals in emerging from the cocoon.

## III Experiments and Discussion of Results

### *Explanation of Table I*

Linkage between *wh* and *st* has been shown by Whiting and Benkert (1934) who reported 9.0 per cent. crossovers in the coupling cross and 12.4 per cent. in the repulsion. Tests with *bk* have indicated linkage with *wh*$^e$ (or *wh*) and *st* as shown by three-point crosses in which *bk*, *wh*$^e$ and *st* were introduced in the four possible ways (Table I). Two of these experiments (III–a and IV–a) gave results obviously affected in some way by modifying factors; these two were therefore repeated (III–b and IV–b).

For each experiment the genotype of the $F_1$ females is given with the factors introduced by the parental females placed above those introduced by the parental males. Thus, in Experiment I, +/+ females were crossed to *bk wh*$^e$ *st* males and the $F_1$ females, +/*bk wh*$^e$ *st*, bred unmated. Frequencies of $F_2$ male offspring are given in vertical columns, followed in each case by *(–)* for straights, *(1)* for crossovers in region 1 *(bk* to *wh*$^e$ or *wh)*, *(2)* for region 2 *(wh*$^e$ or *wh* to *st)* or *(1, 2)* for doubles. Below the totals for each experiment are given the percentages of crossovers in region 1, in region 2 and the percentages of coincidence respectively. Coincidence is determined by the formula:

$$\frac{\text{doubles} \times \text{total} \times 100}{\text{region 1 crossovers} \times \text{region 2 crossovers}}$$

### *Experiments I and II*

In Experiments I and II there are no significant deviations from the expected equality of alternative gametic types except for the relatively low viability of the triple recessive, *bk wh*$^e$ *st*.

This difference is more notable in Experiment II where wild type *(+)* is well in excess. In making the counts in this experiment, cocoons containing pupae which failed to emerge were opened and the phenotype as regards eyes and legs determined. The presence of *bk* can not be detected unless the wings are well expanded. Fifty *wh*$^{e}$ *st* individuals indeterminable for *bk* were added to the triple recessive class.

*Experiment III*

III–a and IV–a gave rather puzzling results. It has already been pointed out that deviations from expected ratios, in the case of these three factors, can not be explained on the basis of masking effects, overlapping or viability differences. In Experiment III–a, one has only to glance at the ratios of the alternative types to see that they have been affected significantly. In every case the *bk* class is reduced, suggesting strongly the presence of a recessive lethal factor linked to *bk* and brought in on the maternal side. If such a lethal exists, with each parental female heterozygous, only one half of her daughters would carry it. In this experiment, eight parental females from the *bk wh*$^{e}$ stock were crossed to a single *st* male. Twenty-three unmated $F_1$ females, *bk wh*$^{e}$*/st,* from these matings were set. A fraternal analysis of the $F_2$ data showed very conclusively that the reduction of the *bk* classes was present in every fraternity, whereas on the basis of chance, if the above postulation were valid, this effect would be present in only half. Furthermore, it would be necessary to assume that the eight parental stock females were all heterozygous for the lethal; this is unlikely, as, if such a factor were present in the stock, only one half or fewer of the females would be carriers. On this basis the chance would be very small indeed that this factor is a simple, "all or none" recessive lethal.

In the face of these facts, the most likely assumption is that each of the stock females was homozygous for a semilethal factor linked to the left of *bk* but too far removed to show linkage with *wh*$^{e}$ or *st*. That such a factor was not brought in by the parental male is shown clearly in the table; if such were the case, one would expect the *st* straights to be lower than the *bk wh*$^{e}$ straights. The reverse is true (*st* 882, *bk wh*$^{e}$ 607). Thus, all of the $F_1$ females were apparently heterozygous for a semilethal to the left of *bk*. Frequency of the class containing *bk* relative to the alternative class was highest for the straights (607/882

or .688), slightly lower for the region 2 crossovers (60/115 or .522), considerably lower for the region 1 crossovers (133/521 or .255) and lowest of all for the doubles (2/27 or .0741). This is what might be expected if crossing over in region 1 and possibly to a lesser extent in region 2 interferes with crossing over between the semilethal and *bk*.

This experiment was repeated (III–b) by making a single parental cross with a female from a different portion of the *bk wh*$^{e}$ stock. As can be seen by comparing the alternative classes in the table, there was no significant reduction and consequently no evidence for the presence of lethals or semilethals.

### *Experiment IV*

Experiment IV–a, in which *wh/wh* females were crossed to two *bk st* males also gave results which can be interpreted only on the basis of a semilethal factor to the left of *bk*. In this case, however, the semilethal was brought in by the parental males which occurred in $F_2$ from III–a and therefore might be expected to carry such a factor. Analysis of the $F_2$ fraternities from these two males shows that both carried a semilethal, although those from one (totaling *bk* 433 to non-*bk* 511 or .847) seem less affected than those from the other (*bk* 187 to non-*bk* 274 or .682). As in III–a, interference is likewise suggested here between region 1 and the region to the left of *bk*. Frequency of the class containing *bk* relative to its alternative is low for region 1 singles (106/161 or .658) and for the doubles (1/7 or .143) while it is higher for the straights (456/562 or .811) and for the region 2 singles (53/55 or .964).

This experiment was also repeated (IV–b) and although the *bk* classes show a slight reduction, there is no striking deviation such as that obtained in III–a and IV–a. If one were to consider the ratios from III–a and IV–a alone, without recourse to the data obtained in Experiments I, II, III–b and IV–b, one would probably be inclined to explain them on the basis of a very unstable viability of individuals carrying *bk*. As has been pointed out, however, the *bk* classes maintain practically equal viability with their alternatives in these latter experiments.

Further evidence for the presence of a semilethal in Experiment IV–a has been obtained by comparing the average number of wasps per vial in this experiment with the average obtained in IV–b. These experiments were made under very similar con-

ditions of temperature, size and number of caterpillars per vial, age of $F_1$ females and time permitted to each female in each vial. On the basis of the first four vials through which each female was passed, those from Experiment IV–a averaged 27.25 males per vial; those from IV–b, 36.25. This suggests the presence of a semilethal in IV–a. In none of the other experiments were conditions similar enough to permit such a comparison.

*Linkage test with gz$^s$*

A further instance of the operation of a modifying factor, again probably a semilethal, was encountered in the $F_2$ from a linkage test in which *bk wh$^c$* stock females were crossed to shiny, *gz$^s$*, males. The data are presented here because of the further suspicion thrown upon the *bk wh$^c$* stock as the carrier of such factors. $F_1$ virgins, *bk wh$^c$/gz$^s$*, gave offspring as follows: *bk wh$^c$* 210, *bk wh$^c$ gz$^s$* 229, + 245, *gz$^s$* 244, *bk* 55, *bk gz$^s$* 57, *wh$^c$* 99, *wh$^c$ gz$^s$* 80, showing independent segregation of *gz$^s$*. Disregarding *gz$^s$*, we get: *bk wh$^c$* 439, + 489, *bk* 112, *wh$^c$* 179. The percentage of recombination between *bk* and *wh$^c$* is 23.9 per cent. The disparity of the *bk* classes (*bk* 551, non-*bk* 668) and lack of disparity in the others (*wh$^c$* 618, non-*wh$^c$* 601; *gz$^s$* 610, non-*gz$^s$* 609) again points to the interpretataion that a linked semilethal to the left of *bk* was introduced from the maternal side. Here again interference is suggested, as *bk* relative to non-*bk* is less frequent among the crossovers (112/179 or .626) than among the straights (439/489 or .898).

Map position of a semilethal can not be determined since the degree of lethality is unknown. As higher degrees are assumed, the factor would be shifted to the left, toward the locus of a complete lethal; as lower degrees are assumed, the factor would more closely approximate the locus of *bk*. That there is an appreciable distance between *bk* and the semilethal or semilethals of the present series of experiments, is evidenced by the disparity of *bk* to non-*bk* ratios among the straights and the crossover classes, which disparity is readily explained by interference.

An alternative hypothesis that the exceptional ratios of Experiments III–a and IV–a are due to the presence of minus modifiers for *bk*, causing overlapping of the character, might be suggested. This is untenable because of the definitely lower average number of offspring per vial in IV–a as compared with IV–b and the fact that extreme *bk* males, consequently without

minus modifiers, were selected from III–a as the male parents for Experiment IV–a.

### IV SUMMARY

1. Broken, *bk*, white *wh*, (or its allele carrot, $wh^c$) and stumpy, *st*, are linked in that order by 25 + or – and 9 + or – crossover units respectively.

2. Three-point crosses involving these factors were made; map distances and ratios of coincidence were determined in each case.

3. Deviations from the expected ratios are due to semilethal factors to the left of *bk*. Frequency of the class containing *bk* relative to the alternative class is highest for the straights and region 2 crossovers as might be expected if region 1 crossovers interfere with crossing over between the semilethal and *bk*.

4. Shiny, $gz^s$, shows no linkage with the white group.

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1934. *Genetics*, 19: 237–267.

## SEXUAL REPRODUCTION AND THE CAROTINOID PIGMENTS IN PLANTS[1]

OUR interest in the carotinoid pigments in relation to the physiology of sexual reproduction was somewhat accidental. It was observed that when certain types of plants, especially soybeans, were brought into reproduction by means of exposure to appropriately short photoperiods, the leaves, despite the reduced amount of light received, became eventually intensely green. Since color of the foliage is due primarily to the chloroplast pigments, determinations were made of the relative concentrations of total chlorophyll, carotene and xanthophyll. The results showed that at the approximate time of anthesis, though not before or later, about the same amount of chlorophyll was present in the reproductive and vegetative plants of a corresponding age, but that the carotene and xanthophyll concentration was conspicuously higher in the leaves of those flowering and setting fruit.

[1] Read at the general session of the American Society of Plant Physiologists, Philadelphia, Pennsylvania, December 30, 1940.

The above observations and some general conclusions made about eight years ago (Murneek, 1934) seemed then somewhat unusual if not far fetched. Corroborative evidence has been presented since by other investigators (Virtanen *et al.*, 1933; Nagasima, 1938).

### Carotinoids in Soybeans

Continuing this study, more detailed assays on the distribution and relative quantities of the carotinoids have been made on reproductive soybean plants, varieties Biloxi and Ilini (Murneek, 1937, 1939). It has been found that the concentration of carotene and xanthophyll reaches a maximum at the period of flowering and then decreases. The reduction of these yellow pigments in the nodes, particularly in presence of fruits (seed pods) suggests that either they are translocated to the rapidly developing reproductive organs or perhaps are changed to some other, possibly vitamin A-like substances, which may have moved into the embryos. We are inclined not to consider the capacity to convert some of the carotinoids (provitamins-A) to vitamin A, an exclusive property of animals.

Repeated attempts, however, to detect vitamin A, or substances that might give a similar reaction with suggested and usable reagents, particularly antimony trichloride, have given so far negative results. This in view of the well-documented evidence that vitamin A apparently is a constituent of the chondriosomes and nucleoli of many plants (Joyet-Lavergne, 1935, 1937). Extracts of cell contents of nodes and flowers in various stages of development have given in some cases, however, unmistakable absorption spectra at 3280 Å. Such absorption was less certain with extracts obtained from fairly well-developed seed pods. The above is the characteristic absorption band for vitamin A. Being encouraged we have attempted to obtain similar spectrophotometric evidence from corn flowers and young fruit but have not succeeded.

It should be stated here that detailed work with the carotinoid pigments is difficult. They are highly susceptible to auto-oxidation in air at room temperature, though the various isomers may vary in this respect. All extractions and separations have to be done in an atmosphere of nitrogen and with the greatest of care in order to obtain quantitative data. In plant tissues these substances may be protected by oxygen receptors present in most cells.

Another phase of our investigations has consisted of repeated attempts to use commercial forms of carotene (mixtures of isomers), β-carotene and vitamin A ("Potenay"), at various concentrations ground in lanolin, on vegetative soybean and *Rudbeckia* plants. The preparations have been applied as paste on stems or/and the undersides of leaves at frequent intervals and over long periods, also as water emulsions to cuttings or plants in water or in Shive's $R_5C_2$ nutrient solutions (*Xanthium*). In no case have we obtained any effect regarding induction of sexual reproduction, though the terminal meristems were subjected in all cases to careful microscopic examination. Neither have we secured any positive results from the water soluble carotinoid *crocin* (prepared by us from dried saffron flowers) when used on cuttings of *Xanthium* or sprayed on undersides of leaves of several other plants.

These experiments have been "crude" indeed in some respects, but we did expect some response in view of the recent evidence on the high potency of some of these substances on algae (Moewus, 1938).

## CROCIN IN CLAMYDOMONAS

Interest among plant physiologists in the carotinoids has been created greatly since the recent spectacular demonstration of their evident relationship to sexual reproduction in *Clamydomonas* (Moewus, 1938, 1939).

*C. eugametos* var. *simplex,* the chief form of the genus used in these experiments, is a single-celled isogamous two-ciliated alga. Motility of the gametes can be induced in darkness by exposure of the organisms to red light. This is due to a photochemical reaction, for the filtrate of the water medium of the irradiated plants will induce motility of gametes without light. The special substance formed and released by *Clamydomonas* in light is *crocin,* $C_{44}H_{64}O_{24}$ (a water soluble carotinoid found in the stigmas of *Crocus sativus* and many other flowers). The quantity produced is extremely small, about 1 mg in 1,000 liters of the filtrate. It was possible to induce motility of the gametes in as weak a concentration of a solution as 1 part of crystalline crocin, isolated from saffron flowers, to 250,000,000,000,000. Repeated tests showed that 5 molecules of crocin could make 4 cells of *Clamydomonas* reactive, which is about 1 molecule per cell!

Though the gametes may be made motile by light of relatively long wave-lengths or by infinitesimal traces of crocin, they are

not able to copulate. A second photochemical reaction must occur, resulting in the production of a specific copulation substance. This may be brought about in darkness by blue or violet (short-wave) light. It is not necessary to expose the organisms themselves to light, for irradiation of the filtrate from cell cultures, which had been previously exposed to red light, will produce this copulation "catalyst." In studying the exposure time required the following startling observations were made: After 24–26 minutes the ♀ cells were made capable to copulate, a longer exposure (35 min.) was non-effective, but a prolonged exposure (74–76 min.) gave the filtrates the property to induce ♂ cells to copulate. This would seem to appear almost a suspiciously strange reaction.

The relation of time of exposure to blue light and induction of sexuality is explained as follows: The substance bringing about induction of copulation in ♀ gametes is a mixture of 3 parts of *cis*–to 1 part of *trans-crocetin dimethyl ester;* of ♂ gametes to copulate, 1 part of *cis*–to 3 parts of *trans-crocetin dimethyl ester.* The cis and trans forms alone are inactive in this respect. The minimum effective dilution is 1: 33,000,000,000. The phenomenon (chemical transformation) has been imitated *in vitro* in Kuhn's laboratory (Kuhn *et al.,* 1939). Moreover, the relative concentrations in a mixture of the two spatial isomers of these esters have been brought in cause and effect relationship with various sex types (relative sexuality) of certain species of *Clamydomonas.*

Still greater variability was introduced into the problem when different forms of the alga were studied. In *C. eugametos* var. *synoica,* with gametes of genotypically undetermined sex, the ♀ determining sex was found to be *picrocrocin,* the bitter substance of saffron flowers, that determining ♂ sex, *safronal,* the odorous substance of saffron.

All the above crocin substances can be traced back to *protocrocin,* to many of the derivatives of which various functions have been ascribed in the sex physiology of *Clamydomonas:* motility, attraction, copulation and sex determination.

Because of the great complexity but definiteness and "elegance" of the results Moewus and Kuhn's investigations have been subjected to severe criticism, especially as regards the statistical reliability of the data. The one by Philip and Haldane (1939) does not refer directly to the physiological side but more specifically to Moewus analyses of genetical behavior of various

races of this alga. Thimann (1940), on the other hand, criticizes chiefly the physiological studies. Considering Kuhn's world-wide reputation as a pigment chemist, it would seem to be advisable not to dismiss these spectacular investigations as unbelievable but study them with circumspection. Verification, of course, would be highly desirable. Moewus may belong to the category of scientists who like to make their studies "beautifully complete." But even if a small part of the results be verified, it would be a notable advance in our understanding of the physiology of sexual reproduction in plants.

### CAROTENES IN FUNGI

Various carotinoids are present in many species of fungi of the *Basidiomycetes, Ascomycetes, Myxomycetes* and *Phycomycetes.* Satina and Blakeslee (1926) observed the occurrence of yellow pigments in the progametes and gametes of *Mucor,* while Chodat and Schopfer (1927) extracted carotene substances from the + (♂) strain of *Mucor heimalis.* In *Allomyces,* a subgenus of *Phycomyces,* an orange pigment is formed in the ♂ cells of the sexual phase. The ♀ cells have no color, though they are similar in structure and motility to the ♂ gametes. This substance was found to be largely γ-carotene with small quantities of the β-form and other isomers in certain strains of this fungus (Emerson and Fox, 1940).

In view of this evidence from the cryptogams one is obliged to agree with recent investigators that the results at least "lend emphasis to the probability that such compounds (carotinoids) play important biological roles in the sexuality and the processes involved in metabolism of reproduction."

### CAROTINOIDS IN HIGHER PLANTS

The presence of yellow and orange pigments in tissues and secretions associated with sexual reproduction of animals and flowering plants has been repeatedly observed by many biologists and biochemists. Studies of these substances in the reproductive organs have indicated the possibility that they may have a physiological function, though at present there is only a cursory information on this subject in reference to higher plants.

Analyses of the quantitative concentration of the carotinoids in seed-bearing plants show two maxima, one during the period of most active growth (post seedling stage) and the second at the time of sexual reproduction (Nagasima, 1938). Their presence

is usually conspicuous in the ♂ reproductive organs (anthers) of most flowers, but the largest total quantities are found in the foliage. In this connection it is rather interesting and possibly significant that the leaf carotenes are to a large extent those that have a provitamin-A activity, viz. α, β and γ forms and cryptoxanthin (MacKinney, 1935). This, of course, does not exclude the possibility that other yellow pigments of this group, even xanthophyll, may not have similar physiological functions. Their interconversion in the plant is highly possible. Fruits and seeds often contain conspicuously large quantities of carotinoids (MacKinney, 1937). With approaching maturity these substances usually decrease in the seeds (Sherman and Salmon, 1939). Leaves of the ♂ plants of the dioecious willow (*Salix fragilis*) have been found to contain more carotene and xanthophyll than those of the ♀ plants. Should this be considered another mark for the male sex?

In conclusion it may be said that interest seems to have been created recently among plant physiologists through current investigations of the carotinoid pigments in general and on their possible specific role in the physiology of reproduction. Studies in this field are still in the preliminary stages requiring the development of new viewpoints and special techniques, but promising interesting results and even startling disclosures.

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## A COLCHICINE–INDUCED TETRAPLOID CABBAGE

CABBAGE seedlings (var. Ferry's Hollander) were treated with a 0.2 per cent. solution of colchicine in the spring of 1940. Upon the cessation of growth and the appearance of hypertrophy the plants were placed in the field to mature. No normal heads formed on any of the plants treated and the majority were grossly abnormal in appearance, some branching dichotomously several times with various bulbous proliferations formed on the stem tips. The plants were taken up in the fall and stored in a cold room until February, 1941, when they were planted in the greenhouse for seed production. Upon the renewal of growth prior to blossoming, all the plants showed similar abnormalities differing only in degree, but cytological examinations of the microsporocytes revealed only two plants of the 22 thus treated to be tetraploid with 18 pairs of chromosomes and one a mixoploid with chromosome numbers varying between 2n and 4n in the same inflorescence. Stomatal measurements and gross external appearances indicated that all the other plants were periclinal chimeras. The two tetraploids were the last to come into flower but produced conspicuously larger flowers and pollen grains than the normal diploid controls.

The tetraploids were selfed and are still producing seeds, and the degree of fertility seems comparable to that of the diploid. Crosses between tetraploid and diploid have resulted in seed to date only when 2n pollen was used.

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